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1:519 U.S. PTO

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February 25, 1998

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RE: New Patent Application in U.S.
Applicant(s): Matthew Todd GILLISPIE et al.
Title: OSTEOCLASTGENIC INHIBITORY AGENT
Atty's Docket: GILLISPIE=1

Sir:

Attached herewith is the above-identified application for Letters Patent including:

- ☒ Specification (58 pages), claims (2 pages) and abstract (1 page)
- ☒ 3 Sheets Drawings (Figures 1-5)
 - ☒ Formal ☐ Informal
- ☒ Declaration and Power of Attorney (2 pages)
 - ☒ Newly executed ☐ Copy from prior application no.
- ☒ Substitute sequence listing and statements in support of filing and submissions in accordance with 37 C.F.R. §1.821-1.825 with diskette
- ☐ Supplemental Preliminary Amendment
- ☒ Information Disclosure Statement with 1449 and 14 references
- ☐ A verified statement to establish small entity status under 37 CFR §1.9 and 37 CFR §1.27 (page(s))
- ☒ A check in the amount of \$ 790.00 (check no. 17937) to cover:
 - ☒ The filing fee calculated as follows:

CLAIMS AS FILED				
FOR	NUMBER FILED	NUMBER EXTRA	RATE	BASIC FEE \$ 790.00
TOTAL CLAIMS	11 - 20=	0	x 22	0
INDEPENDENT CLAIMS	2 - 3=	0	x 82	0
<input type="checkbox"/> Multiple Dependent Claim Presented			x270	
<input type="checkbox"/> Reduction of 1/2 for small entity				- \$
			TOTAL FILING FEE	\$ 790.00

- ☐ Any additional fee required by the filing of an enclosed preliminary or supplemental preliminary amendment has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE	CALCULATION
TOTAL	*	MINUS **	=	x \$ 22.00	\$
INDEP	*	MINUS ***	=	x \$ 82.00	\$
[] Multiple Dependent Claim Presented				x \$270.00	\$
Total of Above Calculations =					\$
Reduction by 1/2 for filing by small entity					- \$
Total Additional Fee =					\$

[] _____

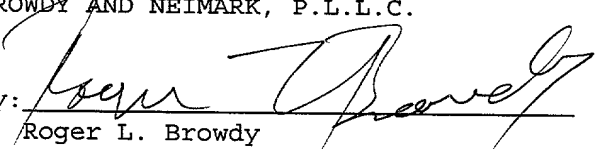
[X] Return Receipt Postcard (in duplicate)

The following statements are applicable:

- [X] The benefit under 35 USC §119 is claimed of the filing date of: Japan Application No. 55468/1997 in Japan on 25 February 1997. A certified copy of said priority document [] is attached [] was filed in progenitor case _____ on _____. Application No. _____ in _____ on _____. A certified copy of said priority document [] is attached [] was filed in progenitor case _____ on _____.
- [] The present application is a [] Continuation [] Divisional [] Continuation-in-part of prior application No. _____.
- [] Incorporation By Reference. The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied herewith, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
- [] A signed statement deleting inventor(s) named in the prior application is attached.
- [] Amend the specification by inserting before the first line the sentence: --This is a __ continuation __ division of copending parent application Serial No. filed _____.--
- [] Certain documents were previously cited or submitted to the Patent and Trademark Office in the following prior application _____, which is relied upon under 35 USC §120. Applicants identify these documents by attaching hereto a form PTO-1449 listing these documents, and request that they be considered and made of record in accordance with 37 CFR §1.98(d). Per Section 1.98(d), copies of these documents need not be filed in this application.
- [] A verified statement claiming small entity status is enclosed in progenitor application no. _____, filed _____. Status is still proper and desired.
- [] The undersigned attorney of record hereby revokes the powers of attorney of:

- [] The undersigned attorney of record hereby appoints associate power of attorney, to prosecute this application and to transact all business in the Patent and Trademark Office in connection therewith to:
- [X] The Commissioner is hereby authorized to charge payment of the following additional fees associated with this communication or credit any overpayments to Deposit Account No. 02-4035:
- [X] Any additional filing fees required under 37 CFR §1.16.
- [X] Any patent application processing fees under 37 CFR §1.17.
- [X] The Commissioner is hereby authorized to charge payment of the following fees, based on any paper filed during the pendency of this application or any CPA thereof, to effect any amendment, petition, or other action requested in said paper or credit any overpayments to Deposit Account No. 02-4035:
- [X] Any patent application processing fees under 37 CFR §1.17.
- [] The issue fee set in 37 CFR §1.18 at or before mailing the Notice of Allowance, pursuant to 37 CFR §1.311(b).
- [X] Any filing fees under 37 CFR §1.16 for presentation of extra claims.
- [X] If a paper is untimely filed in this or any CPA thereof by Applicant(s), the Commissioner is hereby petitioned under 37 CFR. §1.136(a) for the minimum extension of time required to make said paper timely. In the event a petition for extension of time is made under the provisions of this paragraph, the Commissioner is hereby requested to charge any fee required under 37 CFR §1.17 to Deposit Account 02-4035.
- [X] The Commissioner is hereby authorized to credit any overpayment of fees accompanying this paper to Deposit Account No. 02-4035.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.

By: 
Roger L. Browdy
Registration No. 25,618

RLB:bcs

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Art Unit:
)	
Matthew Todd GILLISPIE et al.)	Washington, D.C.
)	
U.S. App. No.:)	
)	February 25, 1998
Filing Date: 25 February 1998)	
)	
For: OSTEOCLASTGENIC...)	Docket No.: GILLISPIE=1

**STATEMENTS IN SUPPORT OF FILING AND SUBMISSIONS
IN ACCORDANCE WITH 37 C.F.R. §1.821-1.825**

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to the examination of the above-described
application, please amend the present application as follows:

IN THE SPECIFICATION

Please substitute the attached Sequence Listing section,
pages 40-55, for the Sequence Listing section as originally filed,
pages 40-58.

IN THE CLAIMS

Please renumber original pages 59-60 as new pages 56-57,
to take into account the additional Sequence Listing section.

IN THE ABSTRACT

Please number the abstract as page 58.

REMARKS

Applicants have added into the present specification a
substitute paper copy Sequence Listing section according to 37

C.F.R. §1.821(c) as new pages 40-55, and have renumbered pages 59-61 as new page numbers 56-58. Furthermore, attached hereto is a 3 1/2" floppy disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

The following statement is provided to meet the requirements of 37 C.F.R. 1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that, on information and belief, the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

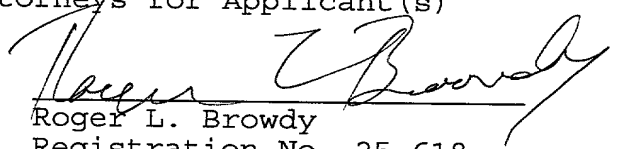
Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
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OSTEOCLASTGENIC INHIBITORY AGENT**Background of the Invention****Field of the Invention**

The present invention relates to an osteoclastgenic inhibitory agent comprising an interleukin-18 (hereinafter abbreviated as "IL-18") or its functional equivalent.

Description of the Prior Art

Osteoblasts' bone formation and osteoclasts' bone resorption are well balanced in healthy living bodies, and this keeps the bone tissues in normal conditions while old bone tissues are being replaced with fresh ones without altering the original bone shape. The phenomenon plays an important role in keeping living bodies' homeostasis such as the controlling of blood calcium concentration within a desired range. Once the balance is lost, especially when the bone resorption level exceeds the bone formation level, bone-related diseases and other diseases may be induced. Therefore, elucidation of the whole mechanism of bone resorption in living bodies, particularly, elucidation of osteoclasts is greatly highlighted due to scientific and clinical significance thereof.

However, the mechanism of osteoclast formation has not yet been completely elucidated even though interleukin 1 as a promoter and interleukin 4 as an inhibitor were found. This is because, similarly as various phenomena in living bodies, osteoclast formation in living bodies is controlled by the close and complicated relationship between promoters and inhibitors.

Based on these, it is greatly expected to establish an effective osteoclastgenic inhibitory agent from the viewpoint of scientific and clinical aspects.

Summary of the Invention

The object of the present invention is to provide a novel and effective osteoclastgenic inhibitory agent. To solve the object the present inventors energetically studied for IL-18, i.e., one of cytokines as communication transferring substances in immune systems, which induces production of interferon- γ (hereinafter abbreviated as "IFN- γ "), an important biologically active substance for immunocompetent cells, and granulocyte/macrophage colony-stimulating factor (hereinafter abbreviated as "GM-CSF"), and augments cytotoxicity and induces formation of killer cells. At the finding, IL-18 was described as an **interferon- γ -inducing factor** as reported by Haruki OKAMURA in Japanese Patent Kokai Nos. 27,189/96 and 193,098/96, and in *Nature*, Vol. 378, No. 6,552, pp. 88-91 (1995), and then called IL-18 according to the proposal of Shimpei USHIO et al., in *The Journal of Immunology*, Vol. 156, pp. 4,274-4,279 (1996).

The present inventors found that a particular gene, capable of inhibiting osteoclast formation from osteoclastic precursor cells *in vitro*, is specifically expressed in quantities in stroma cells derived from mouse myeloma. Their further detailed analysis revealed that (i) the gene encodes IL-18 that includes SEQ ID NO: 7 as a core sequence, (ii) IL-18 and functional equivalents thereof effectively inhibit osteoclast

formation, and (iii) the inhibition is mainly due to the action of GM-CSF induced and produced by IL-18.

Based on these, the present inventors solved the present object by an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient.

Brief Description of the Accompanying Drawings

FIG. 1 shows the structure of the recombinant DNA pKGFHH2.

FIG. 2 shows the structure of the recombinant DNA pCSHIGIF/MUT35.

FIG. 3 shows the structure of the recombinant DNA pCSHIGIF/MUT42.

FIG. 4 shows the structure of the recombinant DNA pBGHuGF.

FIG. 5 shows the structure of the recombinant DNA pKGFMH2.

In these figures, KGFHH2 cDNA means a cDNA encoding the IL-18 according to the present invention: IGIF/MUT35; a DNA encoding the IL-18 according to the present invention: IGIF/MUT42; a DNA encoding the IL-18 according to the present invention: HuIGIF; a chromosomal DNA encoding the IL-18 according to the present invention: KGFMH2 cDNA; a cDNA encoding the IL-18 according to the present invention: 5S; a gene for 5S ribosomal RNA: Ptac; a tac promoter: rrnBT1T2; a termination region of a ribosomal RNA operon: AmpR; an ampicillin resistant gene: pBR322ori; a replication origin of

Escherichia coli: CMV; a cytomegalovirus promoter: IFNss; a nucleotide sequence encoding a signal peptide for subtype $\alpha 2b$ of human interferon- α .

Detailed Description of the Invention

The present invention relates to an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient. The wording "IL-18" as referred to in the invention includes polypeptides with the above property independently of their sources and origins. For example, the IL-18 used in the present invention includes, as internal partial amino acid sequences, the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, as well as SEQ ID NO: 4 and SEQ ID NO: 5, and includes the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 7 as a whole. The wording "functional equivalent(s)" as referred to in the present invention includes (i) those wherein one or more amino acids in the amino acid sequence of IL-18 are replaced with different amino acids, (ii) those wherein one or more amino acids are added to the N- and/or C-termini of the amino acid sequence of IL-18, (iii) those wherein one or more amino acids are inserted into the internal sites of the amino acid sequence of IL-18, (iv) those wherein one or more amino acids in the N- and/or C-terminal regions of the amino acid sequence of IL-18 are deleted, and (v) those wherein one or more amino acids in the internal regions of the amino acid sequence of IL-18 are deleted; all of these modifications should be made within the range that does not

substantially lose the property of osteoclast formation by IL-18 among the inherent property of IL-18. Examples of such functional equivalents are described along with their detailed amino acid sequences in Japanese Patent Application No. 20,906/97 by the same applicant of the present applicant, i.e., polypeptides which are capable of inducing production of interferon-gamma by immunocompetent cells, wherein said polypeptides contain either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs: 1, 2 and 4 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine. The different amino acids to replace the cysteine(s) are not restricted to any types, as far as the resulting polypeptide, containing an amino acid sequence replaced with the different amino acid(s), exhibits an activity of inducing production of IFN- γ by immunocompetent cells in the presence or absence of an appropriate cofactor, as the wild-type polypeptides containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, and a stability significantly higher than that of the wild-type polypeptides. The different amino acids include serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine, among which the most preferable amino acid is serine or alanine. Embodiments of the amino acid sequences, containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, in which one or more cysteines are to be replaced

with different amino acid(s) are the wild-type polypeptides containing SEQ ID NO: 6 or 7. SEQ ID NO: 6 contains cysteines at the 38th, 68th, 76th, and 127th positions from the N-terminus. SEQ ID NO: 7 contains cysteines at the 7th, 75th, and 125th positions. The polypeptides include those containing the amino acid sequence of any one of SEQ ID NOs: 20-26, which are derived from the wild-type polypeptide containing SEQ ID NO: 6, those containing the amino acid sequence of SEQ ID NO: 27 or 28, which are derived from the wild-type polypeptide containing the amino acid sequence of SEQ ID NO: 7, and those containing an amino acid sequence derived from any one of SEQ ID NOs: 20-28 by adding, removing, and/or replacing one or more amino acids to and/or at position(s) excepting the positions where the cysteine(s) have been replaced while retaining the desired biological activities and stability. The wording "one or more amino acids" means the number of amino acids which conventional methods such as site-directed mutagenesis can usually add, remove or replace. The polypeptides containing any one of SEQ ID NOs: 20-28 possess both stability and biological activities significantly higher than those of the wild-type polypeptides.

The functional equivalents as referred to in the present invention further include glycosylated polypeptides of IL-18 and the above polypeptides. Any of these IL-18 and functional equivalents thereof, both of which are included to and referred to as "IL-18" in the present invention, unless specified otherwise, can be used in the present invention independently of their origins; those prepared by separating from natural sources such as cell cultures and from artificially

synthesized ones using recombinant DNA technology and peptide synthesis.

With economical viewpoint, methods of recombinant DNA technology are advantageously used; generally, desired IL-18 can be obtained by introducing DNAs encoding IL-18 into appropriate hosts derived from microorganisms, plants, and animals to form transformants, culturing the transformants in nutrient culture media in a conventional manner, and purifying the cultures by conventional methods used for purifying cytokines. Any DNAs can be used as the above DNAs as long as they contain a DNA encoding IL-18, and can be suitably selected depending on the purpose of the use of the present osteoclastgenic inhibitory agent or on the recombinant DNA technology used. For example, Japanese Patent Kokai Nos. 193,098/96, 231,598/96, and 27,189/96 by the same applicant of the present invention disclose in detail methods for producing IL-18 by culturing transformed microorganisms into which DNAs including a cDNA encoding mouse or human IL-18 are introduced; and Japanese Patent Application No. 185,305/96 by the same applicant of the present invention discloses in detail a method for producing IL-18 encoding human IL-18 by culturing transformed animal cells which have an introduced DNA that includes a chromosomal DNA encodes human IL-18. Japanese Patent Application No. 20,906/97 by the same applicant of the present invention discloses in detail a method for producing IL-18 by culturing transformed animal cells having an introduced DNA which includes a DNA encoding a functional equivalent of human IL-18.

The aforesaid recombinant DNA technology has an

economical advantage, but depending on the hosts and DNA sequences used, the IL-18 thus obtained may have somewhat different physicochemical property from those of IL-18 produced and functions *in vivo*. Japanese Patent Application No. 67,434/96 by the same applicant of the present invention discloses in detail a preparation of IL-18 using established human cell lines as natural sources, and Japanese Patent Application No. 213,267/96 by the same applicant also discloses in detail the preparation using an interleukin-1 β -converting enzyme. The IL-18 obtained by those preparations can be estimated to have substantially the same or equal physicochemical property to that of IL-18 that is produced and functions *in vivo*, and the yield can be estimated to be slightly lower. However, such IL-18 has an advantage that it has a fewer side effects when used as pharmaceuticals directed to administering to warm-blooded animals in general and including humans. When applying purification methods using monoclonal antibodies specific to IL-18, as disclosed in Japanese Patent Application No. 231,598/96 by the same applicant of the present invention, a relatively-high purity IL-18 can be obtained in a minimum labor and cost.

The present osteoclastgenic inhibitory agent comprising the aforesaid IL-18 includes any types and forms usable to inhibit osteoclast formation both *in vivo* and *in vitro*. The present agent can be advantageously used as ingredients for cell culture media for animal cells, which satisfactorily inhibit osteoclast formation, maintain, proliferate, and/or differentiate the desired cells; components

of screening kits for bone-related therapeutic agents; bone-resorption regulatory agents; and agents for osteoclast-related diseases. The bone-resorption regulatory agents include medicaments and health foods that exert an osteoclastgenic inhibitory activity *in vivo*, control bone resorption to normal conditions, and improve unfavorable physical conditions such as a relatively-insignificant arthralgia. The agents for osteoclast-related diseases include medicaments used to prevent and/or treat diseases caused by an excessive osteoclast formation and/or its function. Examples of such diseases are hypercalcemia, osteoclastoma, Behçet's syndrome, osteosarcoma, arthropathy, chronic rheumatoid arthritis, deformity ostitis, primary hyperthyroidism, osteopenia, and osteoporosis. Varying depending on the types of agents and diseases to be treated, the present agent is usually formulated into a liquid, paste, or solid form which contains 0.000002-100 w/w %, preferably, 0.0002-0.5 w/w % of IL-18.

The present osteoclastgenic inhibitory agent can be IL-18 alone or compositions comprising IL-18 and one or more other ingredients such as carriers, excipients, diluents, adjuvants, antibiotics, and proteins such as serum albumin and gelatin as stabilizers; saccharides such as glucose, maltose, maltotriose, maltotetraose, trehalose, sucrose, isomaltose, lactose, panose, erlose, palatinose, lactosucrose, raffinose, fructooligosaccharide, galactooligosaccharide, lentinan, dextrin, pullulan, and sugar alcohols including sorbitol, maltitol, lactitol, and maltotriitol; buffers comprising phosphates or citrates mainly; and reductants such as 2-

mercaptoethanol, dithiothreitol, and reduced glutathione; and optionally biologically active substances such as interferon- α , interferon- β , interferon- γ , interleukin-2, interleukin-3, interleukin-6, interleukin-12, TNF- α , TNF- β , GM-CSF, estrogen, progesterone, chlormadinone acetate, calcitonin, somatokine, somatomedin, insulin-like growth factor, ipriflavone, parathyroid hormone (PTH), norethisterone, busulfan, ancitabine, cytarabine, fluorouracil, tetrahydrofurfuryl fluorouracil, methotrexate, vitamin D₂, active vitamin D, Krestin[®] or polysaccharide K, L-asparaginase, and OK-432 or Picibanil[®]; and calcium salts such as calcium lactate, calcium chloride, calcium monohydrogenphosphate, and L-calcium L-aspartate. When used as agents for administering to warm-blooded animals in general and including humans, i.e., agents for osteoclast-related diseases, the present agent can be preferably formulated into compositions by appropriately combining with one or more of the above physiologically-acceptable substances.

The present osteoclastgenic inhibitory agent includes medicaments in a unit dose form used for administering to warm-blooded animals in general and including humans. The wording "unit dose form" means those which contain IL-18 in an amount suitable for a daily dose or in an amount up to four fold by integers or up to 1/40 fold of the dose, and those in a physically separated and formulated form suitable for prescribed administrations. Examples of such formulations are injections, liquids, powders, granules, tablets, capsules, troches, collyriums, nebulas, and suppositories.

The present agent as an osteoclastgenic inhibitory

agent effectively treat and prevent osteoclast-related diseases independently of oral and parenteral administrations. Varying depending on the types and symptoms of patients' diseases, the present agent can be administered to the patients orally, intradermally, subcutaneously, muscularly, or intravenously at a dose of about 0.5 µg to 100 mg per shot, preferably, at a dose of about 2 µg to 10 mg per shot of IL-18, 2-6 fold a day or 2-10 fold a week for one day to one year.

In the below, with reference to experiments, the preparation, physicochemical property, and biological activity of the IL-18 according to the present invention are described:

Experiment 1

Preparation of human IL-18

According to the method in Japanese Patent Kokai No. 231,598/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, **pKGFHH2**, linked to a cDNA encoding human IL-18, was prepared. Dideoxyribonucleotide sequencing analyzed that, as shown in FIG. 1, in the recombinant DNA, KGFHH2 cDNA containing the base sequence of SEQ ID NO: 8 was linked to the downstream of Ptac, a Tac promoter. The recombinant DNA pKGFHH2 contained the amino acid sequences of SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 8.

According to the method in Japanese Patent Kokai No. 231,598/96, the recombinant DNA pKGFHH2 was introduced into an *Escherichia coli* Y1090 strain, ATCC 37197, and the strain was cultured. The produced polypeptide was purified by

immunoaffinity chromatography to obtain a purified human IL-18 with a purity of at least 95% in a yield of about 25 mg/ℓ culture. According to the method in Japanese Patent Kokai No. 193,098/96 by the same applicant of the present invention, the purified human IL-18 was analyzed for biological activity and physicochemical property as indicated below: When culturing human lymphocytes, collected by a conventional manner from a healthy donor, in the presence of the purified human IL-18, IFN- γ production was observed depending on the concentration of IL-18, resulting in a confirmation that IL-18 has an activity of inducing IFN- γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified IL-18 was subjected to SDS-PAGE, resulting in a major band with an IFN- γ inducing activity at a position corresponding to 18,500 \pm 3,000 daltons. The IL-18 gave a pI of 4.9 \pm 1.0 as determined by conventional chromatofocusing. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the IL-18 had the amino acid sequence of SEQ ID NO: 9, i.e., the amino acid sequence of SEQ ID NO: 8 where a methionine residue was linked to the N-terminus.

Experiment 2

Preparation of human IL-18

According to the method in Japanese Patent Application No. 67,434/96 by the same applicant of the present invention, THP-1 cells, ATCC TIB 202, a human monocyte cell line derived from a male with acute monocytic leukemia, were inoculated to

the dorsum subcutaneous tissues of new born hamsters, followed by feeding the hamsters for three weeks. Tumor masses, about 15 g weight each, formed in the subcutaneous tissues of each hamster, were extracted, dispersed in media, and disrupted. The polypeptide obtained from the disrupted cells was purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of an about 50 ng/head.

Similarly, according to the method in Japanese Patent Application No. 67,434/96, the purified human IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that culturing human lymphocytes, collected from healthy donors in a conventional manner, in the presence of different concentrations of the human IL-18, resulted in an IL-18 dose-dependent IFN- γ production. This revealed that the human IL-18 has a biological activity of inducing IFN- γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE using 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- γ production inducing activity at a position corresponding to 18,000-19,500 daltons. According to the peptide map disclosed in Japanese Patent Application No. 67,434/96, the human IL-18 was treated with clostripain commercialized by Sigma Chemical Company, Missouri, USA, to obtain polypeptide fragments, followed by subjecting the fragments for fractionation to high-performance liquid chromatography (HPLC) using "ODS-120T", a column commercialized

by Tosoh Corporation, Tokyo, Japan, and analyzing the amino acid sequences of the fragments from the N-terminus to reveal the following amino acid sequences of SEQ ID NOs: 10 to 13. These amino acid sequences were completely coincided with amino acids 148-157, 1-13, 45-58, and 80-96 in SEQ ID NO: 6. The data shows that the human IL-18 obtained in Experiment 2 has the amino acid sequence of SEQ ID NO: 6 and all the partial amino acid sequences of SEQ ID NOs: 1 to 5.

Experiment 3

Preparation of functional equivalents

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA, pCSHIGIF/MUT35, was linked to a DNA encoding a functional equivalent of human IL-18 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. Dideoxyribonucleotide sequence analysis revealed that as shown in FIG. 2, in the recombinant DNA, DNA IGIF/MUT35 with SEQ ID NO: 14 linked to the downstream of a base sequence encoding a signal peptide of subtype $\alpha 2b$ in human interferon- α in the same reading-frame, as reported by K. Henco et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 14, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. The recombinant DNA contained a nucleotide which encodes all the amino acid

sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine at amino acid 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 14.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT35 was introduced into COS-1 cells, ATCC CRL 1650, an established cell line derived from SV40 transformed African Green monkey kidney, followed by culturing the transformed cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 40 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When culturing KG-1 cells, ATCC CCL 246, an established cell line derived from human acute myelogenous leukemia, in the presence of different concentrations of the purified functional equivalent of human IL-18, IFN- γ production was observed depending on the concentration of the IL-18, revealing that the IL-18 has a biological activity of inducing IFN- γ production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- γ production inducing

activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 14.

Experiment 4

Preparation of functional equivalent

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA, pCSHIGIF/MUT42, which was linked to a DNA encoding for a functional equivalent of human IL-18 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. Dideoxyribonucleotide sequencing revealed that, as shown in FIG. 3, in the recombinant DNA, DNA IGIF/MUT42 with SEQ ID NO: 16 linked to the downstream of a base sequence encoding a signal peptide for subtype $\alpha 2b$ of human interferon- α in the same reading frame, as reported by K. Henco et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 16, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. The recombinant DNA contained a nucleotide sequence which encodes all the amino acid sequences of SEQ ID NOs: 1 to

4 and the one of SEQ ID NO: 5 where cysteine 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 16.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT42 was introduced into COS-1 cells, followed by culturing the cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 20 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When cultured KG-1 cells in the presence of different concentrations of the purified functional equivalent, a dose-dependent IFN- γ production was observed, and this revealed that the functional equivalent has a biological activity of inducing IFN- γ production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- γ inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO:

15 which completely corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 16.

Experiment 5

Preparation of human IL-18

According to the method in Japanese Patent Application No. 185,305/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, pBGHuGF, linked to a chromosomal DNA encoding human IL-18, was obtained. Dideoxyribonucleotide sequencing analysis revealed that as shown in FIG. 4, in the recombinant DNA, a chromosomal DNA, which encodes human IL-18, i.e., DNA HuIGIF with SEQ ID NO: 17, was linked to the downstream of a restriction site by a restriction enzyme, *Hind* III. As shown in SEQ ID NO: 17, the chromosomal DNA HuIGIF consists of 11,464 bp where the exon was fragmented by four introns positioning at nucleotides 83-1,453, 1,466-4,848, 4,984-6,317, and 6,452-11,224. Among the resting nucleotide sequence excluding these introns, nucleotides 3-11,443 from the 5'-terminus are the part that encodes a precursor of human IL-18, and nucleotides 4,866-4,983 are the part that encodes an active human IL-18. The chromosomal DNA contained nucleotides sequences encoding SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 4,911-4,928, 4,953-4,970, 11,372-11,392, 6,350-6,364, and 6,413-6,427 in SEQ ID NO: 17.

According to the method in Japanese Patent Application No. 185,305/96, the recombinant DNA pBGHuGF was introduced into CHO-K1 cells, ATCC CCL 61, an established cell line derived from Chinese hamster ovary, followed by culturing the cells. The

culture supernatant was contacted with a supernatant of cell disruptant prepared from a THP-1 cell culture to produce a polypeptide which was then purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of about 15 mg/l culture. According to the method in Japanese Patent Application No. 185,305/96, the polypeptide was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that human lymphocytes, which were collected from a healthy donor, produced IFN- γ depending on the purified human IL-18 concentration when cultured at different concentrations of the human IL-18, revealing that the human IL-18 has a biological activity of inducing IFN- γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- γ inducing activity at a position corresponding to 18,000-19,500 daltons. The N-terminal region of the human IL-18 contained the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region of SEQ ID NO: 17 for an active IL-18.

Experiment 6

Preparation of mouse IL-18

To a 0.5-ml reaction tube were added 8 μ l of 25 mM magnesium chloride, 10 μ l of 10 x PCR buffer, one μ l of 25 mM dNTP mix, one μ l of 2.5 units/ μ l of amplitaq DNA polymerase, one ng of a recombinant DNA, which encodes mouse IL-18 having the

nucleotide sequence of SEQ ID NO: 18 and the amino acid sequence of SEQ ID NO: 7, prepared from a phage DNA clone according to the method in Japanese Patent Kokai No. 27,189/96, and adequate amounts of a sense and antisense primers having nucleotide sequences represented by 5'-ATAGAATTCAAATGAACTTTGGCCGACTTCACTG-3' and 5'-ATAAAGCTTCTAACTTTGATGTAAGTT-3', respectively, which were chemically synthesized based on the amino acid sequences nearness to the N- and C-termini of SEQ ID NO: 7, and the mixture solution was brought up to a volume of 100 µl with sterilized distilled water. The solution thus obtained was subjected in a usual manner to PCR reaction of the following three cycles of successive incubations at 94°C for one minute, 43°C for one minute, and 72°C for one minute, and further 40 cycles of successive incubations at 94°C for one minute, 60°C for one minute, and 72°C for one minute.

The product obtained by the PCR reaction and "pCR-Script SK (+)", a plasmid vector commercialized by Stratagene Cloning Systems, California, USA, were in a conventional manner ligated together using a DNA ligase into a recombinant DNA which was then introduced into "XL-1 Blue MRF'Kan", an *Escherichia coli* strain commercialized by Stratagene Cloning Systems, California, USA., to obtain a transformant. The transformant was inoculated to L-broth (pH 7.2) containing 50 µg/ml ampicillin, followed by the incubation at 37°C for 18 hours under shaking conditions. The culture was centrifuged to obtain the proliferated transformants which were then treated with a conventional alkali-SDS method to isolate a recombinant DNA. A portion of the recombinant DNA isolated was analyzed by

dideoxyribonucleotide sequencing, revealing that the recombinant DNA contained restriction sites of *Eco* RI and *Hind* III at the 5'- and 3'-termini of SEQ ID NO: 18, respectively, and a DNA containing a methionine codon for initiating polypeptide synthesis and a TAG codon for terminating polypeptide synthesis, which were located in just before and after the N- and C-termini of the amino acid sequence as shown in parallel in SEQ ID NO: 18. The recombinant DNA contained the nucleotide sequences of SEQ ID NOs: 1 to 5. These amino acid sequences were encoded by nucleotides 46-63, 85-102, 394-414, 148-162, and 211-225 in SEQ ID NO: 18.

The remaining portion of the recombinant DNA was in a conventional manner cleaved with restriction enzymes of *Eco* RI and *Hind* II, and the resulting 0.1 µg of an *Eco* RI-*Hind* III DNA fragments, obtained by using "DNA LIGATION KIT VER 2", a DNA ligation kit commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, and 10 ng of pKK223-3, a plasmid vector commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been cleaved with a restriction enzyme were linked together, by incubating at 16°C for 30 min to obtain an autonomously-replicable recombinant DNA, **pKGFMH2**. Using competent cell method, an *Escherichia coli* Y1090 strain, ATCC 37197, was transformed using the recombinant DNA pKGFMH2, and the resulting transformant, KGFMH2, was inoculated to L-broth (pH 7.2) containing 50 µg/ml ampicillin, and cultured at 37°C for 18 hours under shaking conditions. The culture was centrifuged to collect the proliferated transformants, followed by applying a conventional SDS-alkali method to a portion of the transformants

to extract the recombinant DNA pKGFMH2. Dideoxyribonucleotide sequencing analysis revealed that, as shown in FIG. 5, KGFMH2 cDNA containing the nucleotide sequence of SEQ ID NO: 18 was linked to the downstream of the Tac promoter in the recombinant DNA pKGFMH2.

Ampicillin was added to L-broth (pH 7.2), which had been sterilized by autoclaving, to give a concentration of 50 µg/ml, cooled to 37°C, and inoculated with the transformant KGFMH2, followed by the culture at 37°C for 18 hours. Eighteen liters of a fresh preparation of the same culture medium was placed in a 20-l jar fermenter, similarly sterilized as above, admixed with ampicillin, cooled to 37°C, and inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 37°C for 8 hours under aeration-agitation conditions. The resulting culture was centrifuged to collect the cultured cells which were then suspended in a mixture solution (pH 7.3) containing 150 mM sodium chloride, 16 mM disodium hydrogenphosphate, and 4 mM sodium dihydrogenphosphate, disrupted by ultrasonication, and centrifuged to remove cell disruptant, and this yielded an about two liters of a supernatant.

To an about two liters of the supernatant was added 10 mM phosphate buffer (pH 7.3) containing ammonium sulfate to give a 40% ammonium saturation. The resulting sediment was removed by centrifugation, and the supernatant was mixed with ammonium sulfate to give an 85% ammonium saturation, allowed to stand at 4°C for 18 hours, and centrifuged at about 8,000 rpm for 30 min to obtain a newly formed sediment. The sediment thus

obtained was dissolved in 10 mM phosphate buffer (pH 6.6) containing 1.5 M ammonium sulfate to give a total volume of about 1,300 ml, and the solution was filtered, and fed to a column packed with about 800 ml of "PHENYL SEPHAROSE CL-6B", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, followed by washing the column with a fresh preparation of the same buffer and feeding to the column a linear gradient buffer of ammonium sulfate decreasing from 1.5 M to 0 M in 10 mM phosphate buffer (pH 6.6) at an SV (space velocity) 1.5. Fractions eluted at around 1 M ammonium sulfate were pooled, concentrated using a membrane filter, and dialyzed against 10 mM phosphate buffer (pH 6.5) at 4°C for 18 hours. The dialyzed solution was fed to a column packed with about 55 ml of "DEAE-5PW", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with 10 mM phosphate buffer (pH 6.5). The column was washed with a fresh preparation of the same buffer, and fed with a linear gradient buffer of sodium chloride increasing from 0 M to 0.5 M in 10 mM phosphate buffer (pH 6.5) at SV 5.5, followed by collecting fractions eluted at around 0.2 M sodium chloride. Thereafter, the fractions were pooled and concentrated similarly as above up to give an about nine milliliters, followed by dialyzing the concentrate against PBS (phosphate buffered saline) at 4°C for 18 hours, and feeding the dialyzed solution to a column packed with "SUPERDEX 75", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with a fresh preparation of the same PBS. The column was fed with a fresh preparation of the same PBS to collect fractions

with an IFN- γ inducing activity, and the fractions were pooled and concentrated with a membrane filter to obtain a purified mouse IL-18 in a yield of about 350 $\mu\text{g}/\ell$ culture.

According to the method in Japanese Patent Kokai No. 27,189/96, the purified mouse IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: Culturing mouse spleen cells, collected by a conventional manner, under different concentrations of the mouse IL-18 resulted in an IFN- γ production depending on the concentrations of the mouse IL-18, and this revealed that the mouse IL-18 has an activity of inducing IFN- γ production by spleen cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE under non-reducing conditions, resulting in a major band with an IFN- γ inducing activity at a position corresponding to 19,000 \pm 5,000 daltons. The N-terminal region of the mouse IL-18 contained the amino acid sequence of SEQ ID NO: 19 which corresponded to the N-terminal region of SEQ ID NO: 18.

With reference to Experiment 7, the biological activity of the IL-18 according to the present invention will be described in more detail, and Experiment 8 describes the cytotoxicity of the IL-18:

Experiment 7

Biological activity

Experiment 7-1

Induction of GM-CSF production

Using a heparinized syringe, blood was collected from

a healthy volunteer and diluted two fold with serum-free RPMI 1640 medium (pH 7.4). The diluent was overlaid on a ficoll and centrifuged, and the collected lymphocytes were washed with RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, and suspended in a fresh preparation of the same medium to give a cell density of 1×10^6 cells/ml, followed by distributing the cell suspension to a 12-well microplate by two ml/well.

Using RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, an IL-18 preparation obtained by the method in Experiment 1 was prepared into a one $\mu\text{g/ml}$ solution which was then distributed to the above microplate by 20-200 $\mu\text{l/well}$. To the microplate was further added a fresh preparation of the same buffer, supplemented with 500 $\mu\text{l/ml}$ of Concanavalin A, by 10 $\mu\text{l/well}$, followed by the incubation at 37°C for 48 hours in a 5 v/v % CO_2 incubator. After completion of the culture, supernatants in each well were sampled by 0.1 ml/well, and determined for GM-CSF content using a conventional enzyme immunoassay. In parallel, a culture system free of IL-18 as a control was provided and treated similarly as above. The data is in Table 1:

Table 1

IL-18* (nM)	GM-CSF yield (pg/ml)
0	510
0.7	2,150
2.8	3,050
5.6	3,950

Note: The symbol "*" means that IL-18 was added to the culture system in the presence of 2.5 µg/ml of Concanavalin A.

The results in Table 1 indicate that lymphocytes as an immunocompetent cell produced GM-CSF depending on the concentration of IL-18 when contacted with IL-18 in the presence of Concanavalin A as a cofactor. It was also confirmed that all of the IL-18 preparations and functional equivalents thereof, which were obtained by the methods in Experiments 2 to 5, induced GM-CSF production even when used alone similarly as above. An IL-18 preparation obtained by the method in Experiment 6 was tested in accordance with Experiment 7-1 except that the human lymphocytes used in the experiment were replaced with spleen cells prepared from mouse by a conventional manner, revealing that the IL-18 preparation also induced GM-CSF production.

Experiment 7-2

Inhibition of osteoclast formation

Experiment 7-2(a)

As reported by T. J. Martin and K. W. Ng in *Journal of Cellular Biochemistry*, Vol. 56, pp. 357-366 (1994), it is considered requisite for contacting osteoclastic precursor cells, derived from hematopoietic stem cells, with osteoblasts or bone marrow stromas to generally differentiate osteoclastic precursor cells into mature osteoclasts. As described by G. D. Roodman in *Endocrine Reviews*, Vol. 17, No. 4, pp. 308-332 (1996), it is generally recognized that osteoclasts have characters of multinucleated cells, tartaric acid-resistant acid

phosphatase (hereinafter abbreviated as "TRAP") activity, and a calcitonin receptor. In a co-culture system of osteoblasts and bone marrow cells as reported by Nobuyuki UDAGAWA et al., in *Journal of Experimental Medicine*, Vol. 182, pp. 1,461-1,468 (1995), these cells respond to factors such as $1\alpha,25$ -dihydroxyvitamin D_3 , prostaglandin E_2 , adrenocortical hormone, interleukin 1, interleukin 6, and interleukin 11, to form osteoclast-like cells (hereinafter may be abbreviated as "OCL"). The formed OCL has characters of osteoclasts *in vivo*. Therefore, the co-culture system well reflects *in vitro* the processes of osteoclast formation *in vivo*. Using this system, experiments for osteoclast formation and osteoclastogenic inhibitory agents can be carried out.

The osteoclastogenic inhibitory activity of the IL-18 according to the present invention was studied using the above co-culture system. The osteoblasts used in this experiment were prepared in a conventional manner by treating a newborn mouse calvaria with 0.1 w/v % collagenase commercialized by Worthington Biochemical Co., Freefold, Australia, and 0.2 w/v % dispase commercialized by Godo Shusei Co., Ltd., Tokyo, Japan. The bone marrow cells were prepared from a mature mouse in a conventional manner. As a negative control, 2×10^4 cells of a primary cell culture of osteoblasts and 5×10^5 cells of bone marrow cells were co-cultured in each well of a 48-well microplate containing 0.4 ml/well of α -MEM medium supplemented with 10 v/v % fetal calf serum (hereinafter designated as "Medium" throughout Experiment 4-2) at 37°C for seven days in a 5 v/v % CO_2 incubator. As a positive control, the above two-

types of cells were co-cultured similarly as in the negative control except that they were cultured in other wells containing 10^{-8} M of $1\alpha,25$ -dihydroxyvitamin D_3 commercialized by Wako Pure Chemicals, Tokyo, Japan, and 10^{-7} M of prostaglandin E_2 commercialized by Sigma Chemical Company, Missouri, USA. The aforesaid two-types of cells were co-cultured similarly as in the positive control except that they were cultured in other wells containing $1\alpha,25$ -dihydroxyvitamin D_3 commercialized by Wako Pure Chemicals, Tokyo, Japan, and prostaglandin E_2 commercialized by Sigma Chemical Company, Missouri, USA., in the same concentrations as used in the positive control, and a concentration of 0.01-10 ng/ml of an IL-18 preparation prepared by the method in Experiment 6. In every co-culture system, the media in each well were replaced with fresh preparations of the same media used in the co-culture systems on the 3rd day after the initiation of each culture. According to the method by Nobuyuki UDAGAWA in *Journal of Experimental Medicine*, Vol. 182, pp. 1,461-1,468 (1995), the cells on the 6th day after the initiation of each culture were fixed and stained based on TRAP activity, followed by counting the stained cells (hereinafter called "TRAP-positive cells") per well. Throughout Experiment 4-2, quadruplet wells under the same conditions were provided for each co-culture system, and the mean value for the TRAP-positive cells per well in each system was calculated. The results are in Table 2:

IL-18 (ng/ml)	Osteoclastgenic formation factor*1	Number of TRAP-positive cells per well*2
0	-	2
0	+	110
0.01	+	114
0.1	+	111
0.5	+	106
1	+	63
2	+	29
4	+	12
8	+	2
10	+	2

Note: *1: The symbols of "+" and "-" show co-culture systems with and without 10^{-8} M $1\alpha,25$ -dihydroxyvitamin D_3 and 10^{-7} M prostaglandin E_2 , respectively.

*2: It shows a mean value of the data from quadruplet wells cultured under the same conditions.

As shown in Table 2, the formation of TRAP-positive cells was not substantially observed in the negative control, but the distinct formation was observed in the positive control. In the co-culture systems, i.e., the positive control supplemented additionally with IL-18, the formation of TRAP-positive cells was inhibited depending on the concentration of IL-18, and the maximum inhibition, i.e., a level equal to that in the negative control, was found at eight ng/ml or more of IL-18. These data strongly indicates that IL-18 has a concrete activity of inhibiting OCL formation *in vitro* and also inhibits osteoclast formation.

Experiment 7-2(b)

As described hereinbefore, it was confirmed that there exist factors that induce the formation of osteoclast-like cells in the co-culture systems used throughout Experiment 7-2. Therefore, in this Experiment 7-2(b), it was studied whether the inhibitory activity of IL-18 on osteoclast formation observed in Experiment 7-2(a) was specific to some factors or not; the osteoclast-like cells were cultured by the same method as used in the negative control in Experiment 7-2(a) except for using a medium supplemented with 10^{-8} M $1\alpha,25$ -dihydroxyvitamin D₃, 10^{-7} M prostaglandin E₂, 200 ng/ml parathyroid hormone, 100 ng/ml interleukin 1, or 20 ng/ml interleukin 11. These culture systems were for positive controls. In parallel, the cells were cultured in other wells by the same method used in the positive controls except for using a medium containing 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, in addition to any one of the above factors at the same

concentration. After completion of the cultures, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The results are in Table 3:

Table 3

Osteoclast formation factor*1 (concentration)	IL-18*2	Number of TRAP-positive cells per well*3
D ₃ (10 ⁻⁸ M)	-	94
	+	3
PGE ₂ (10 ⁻⁷ M)	-	77
	+	3
PTH (200 ng/ml)	-	63
	+	3
IL-11 (100 ng/ml)	-	84
	+	3
IL-1 (20 ng/ml)	-	71
	+	3

Note: *1: D₃, PGE₂, PTH, IL-11, and IL-1 are respectively 1 α ,25-dihydroxyvitamin D₃, prostaglandin E₂, parathyroid hormone, interleukin-11, and interleukin-1 which were added to wells to give the concentrations as indicated in parentheses.
 *2: The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 was not added to.
 *3: It shows a mean value of the data from quadruplet wells cultured under the same conditions.

As shown in Table 3, a distinct formation of TRAP-positive cells was observed in every positive control, but the formation was almost completely inhibited in the presence of IL-18. This strongly indicates that IL-18 has a wide and general activity of inhibiting osteoclast formation independently of osteoclast-formation-related factors.

Experiment 7-2(c)

It was studied whether the osteoclastogenic inhibition by IL-18, confirmed in Experiments 7-2(a) and 7-2(b), was caused by the action of the IL-18-induced GM-CSF. For positive and negative controls, the same co-culture systems employed in Experiment 7-2(a) were used. Using other wells, the co-culture of osteoblasts and bone marrow cells was carried out similarly as the method used for the positive controls except for using a medium supplemented with $1\alpha,25$ -dihydroxyvitamin D_3 and prostaglandin E_2 at the same concentrations used in the positive control, and with (i) 10 μ g/ml of an anti-mouse GM-CSF polyclonal antibody commercialized by R&D Systems, Minnesota, USA, (ii) 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, (iii) (ii) plus 10 μ g/ml of an anti-mouse polyclonal antibody, (iv) 0.1 ng/ml of a mouse GM-CSF commercialized by R&D Systems, Minnesota, USA, or (v) (iv) plus 10 μ g/ml of an anti-mouse GM-CSF polyclonal antibody. After completion of the culture, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The data is shown in Table 4 where the symbols "i" to "v" coincide with those used in the co-culture systems other than the control systems.

Table 4

Culture system*1	Osteoclastogenic factor*2	IL-18*3	GM-CSF*4	Anti-GM-CSF antibody*5	Number of TRAP-positive cells per well*6
N	-	-	-	-	3
P	+	-	-	-	122
i	+	-	-	+	112
ii	+	+	-	-	3
iii	+	+	-	+	111
iv	+	-	+	-	4
v	+	-	+	+	106

Note:

*1; where the symbols "N" and "P" mean negative and positive controls, respectively, and the symbols "i" to "v" correspond to those in the five types co-culture systems used.

*2; where the symbol "+" means that 1 α ,25-dihydroxyvitamin D₃ and prostaglandin E₂ were respectively added to a well to give respective concentrations of 10⁻⁸M and 10⁻⁷M, and the symbol "-" means that these compounds were not added to.

*3; The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 was not added to.

*4; The symbol "+" means that GM-CSF was added to a well to give a concentration of 0.1 ng/ml, and the symbol "-" means that GM-CSF was not added to.

*5; The symbol "+" means that an anti-GM-CSF polyclonal antibody was added to a well to give a concentration of 10 μ g/ml, and the symbol "-" means that the polyclonal antibody was not added to.

As shown in Table 4, the formation of TRAP-positive cells was almost completely inhibited by IL-18, cf., the co-culture system (ii), but the inhibition was almost completely inhibited by the addition of the anti-mouse polyclonal antibody, cf., the co-culture system (iii). Mouse GM-CSF exhibited an activity of inhibiting the formation of TRAP-positive cells similar to IL-18, cf., the co-culture system (iv), and the inhibition was almost completely inhibited by the addition of the anti-mouse GM-CSF polyclonal antibody, cf., the co-culture system (v). The sole use of the anti-mouse GM-CSF polyclonal antibody gave no influence on the formation of TRAP-positive cells, cf., the co-culture system (i). These data strongly indicates that the osteoclastogenic inhibition by IL-18 was due to the action of the IL-18-induced GM-CSF.

Experiment 8

Acute toxicity test

Eight-week-old mice were in a conventional manner injected percutaneously, orally, or intraperitoneally with either of IL-18 preparations obtained by the methods in Experiments 1 to 6. The results showed that these IL-18 preparations had an LD₅₀ of about one mg/kg or more in mice independent of the route of administration. The data evidences that IL-18 can be incorporated into pharmaceuticals for warm-blooded animals in general and including humans without causing no serious side effects.

As described in *Nikkei Biotechnology Annual Report 1996*, pp. 498-499 (1995), published by Nikkei BP Publisher, Tokyo, Japan (1995), the IL-18-induced GM-CSF has not yet been

clinically used in Japan, but applied clinically in USA and Europe. The fact would show that IL-18 has substantially no serious side effects. These facts indicate that the osteoclastgenic inhibitory agent according to the present invention can be successively administered to warm-blooded animals in general and including humans to induce osteoclast formation and exert a satisfactory therapeutic and/or prophylactic effect on osteoclast-related diseases without causing serious side effects.

The following Examples describe the present osteoclastgenic inhibitory agent according to the present invention:

Example 1

Liquid

Either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in physiological saline containing one w/v % human serum albumin as a stabilizer to give a concentration of two mg/ml of the IL-18 preparation. The resulting solutions were in a conventional manner membrane filtered for sterilization into liquids.

The liquids have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of an injection, ophthalmic solution, or collunarium for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 2

Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % purified gelatin as a stabilizer. The solutions thus obtained were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 3

Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % trehalose as a stabilizer. The solutions were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 4

Ointment

"HIVIS WAKO GEL[®] 104", a carboxyvinylpolymer

commercialized by Wako Pure Chemical Industries, Ltd., Tokyo, Japan, and a high-purity trehalose were dissolved in a sterilized distilled water to give respective concentrations of 1.4 w/w % and 2.0 w/w %, and the solution was mixed to homogeneity with either of IL-18 preparations obtained by the methods in Experiments 1 to 6, and adjusted to pH 7.2 to obtain a paste containing about one mg of an IL-18 preparation per g of the product.

Each product thus obtained has a satisfactory spreadability and stability and can be arbitrarily used as an agent in the form of an ointment for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 5

Tablet

"FINETOSE[®]", an anhydrous crystalline α -maltose powder commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, was mixed to homogeneity with either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, and "LUMIN" or 1-1'-1"-triheptyl-11-chinolyl(4)•4•4'-penthamethinchynocyanine-1,1"-dijodide. The mixtures were in a conventional manner tabletted to obtain tablets, about 200 mg weight each, containing an about two milligrams of either of the IL-18 preparations and an about two milligrams of LUMIN per tablet.

The products have a satisfactory swallowability, stability, and cell-activating activity and can be arbitrarily used as agents in the form of a tablet for regulating bone

resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

As described above, the osteoclastgenic inhibitory agent according to the present invention effectively inhibits osteoclast formation. Therefore, the agent can be arbitrarily used as an ingredient for cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Thus the present invention with these useful activities and functions is a significant invention that would greatly contribute to this field.

While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

SEQUENCE LISTING

(1) INFORMATION FOR SEQ ID NO: 1:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 6 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asn Asp Gln Val Leu Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 6 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Phe Glu Asp Met Thr Asp
1 5

(3) INFORMATION FOR SEQ ID NO: 3:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 7 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Phe Lys Leu Ile Leu Lys Lys
1 5

(4) INFORMATION FOR SEQ ID NO: 4:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 5 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Tyr Lys Asp Ser
1 5

(5) INFORMATION FOR SEQ ID NO: 5:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 5 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Thr Leu Ser Cys
1 5

(6) INFORMATION FOR SEQ ID NO: 6:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 157 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60
Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65 70 75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(7) INFORMATION FOR SEQ ID NO: 7:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 157 amino acids

(B)TYPE: amino acid
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn	
1				5					10					15		
Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met	
			20					25					30			
Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile	
		35					40					45				
Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser	
	50					55					60					
Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile	
65					70					75					80	
Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser	
				85					90					95		
Asp	Leu	Ile	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu		
			100				105					110				
Phe	Glu	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Cys	Gln	Lys	Glu		
		115				120					125					
Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp	
	130					135					140					
Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser				
145					150					155						

(8)INFORMATION FOR SEQ ID NO: 8:

(i)SEQUENCE CHARACTERISTICS:
(A)LENGTH: 471 base pairs
(B)TYPE: nucleic acid
(C)STRANDEDNESS: double
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA

(vi)ORIGINAL SOURCE:
(A)ORGANISM: human
(G)CELL TYPE: liver

(ix)FEATURE:
(A)NAME/KEY: mat peptide
(B)LOCATION: 1..471
(C)IDENTIFICATION METHOD: E

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1			5					10					15			
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
		20					25					30				
ATG	ACT	GAT	TCT	GAC	TGT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144

Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile		
		35					40					45					
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192	
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile		
		50				55					60						
TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240	
Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile		
65					70					75					80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys		
				85					90					95			
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys		
				100				105					110				
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384	
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu		
		115					120					125					
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432	
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu		
		130				135					140						
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471	
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp					
145					150					155							

(9) INFORMATION FOR SEQ ID NO: 9:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 11 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1				5					10	

(10) INFORMATION FOR SEQ ID NO: 10:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 10 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: C-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp
1				5				10	

(11) INFORMATION FOR SEQ ID NO: 11:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 13 amino acids
 (B)TYPE: amino acid
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg
1 5 10

(12) INFORMATION FOR SEQ ID NO: 12:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 14 amino acids
 (B)TYPE: amino acid
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg
1 5 10

(13) INFORMATION FOR SEQ ID NO: 13:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 17 amino acids
 (B)TYPE: amino acid
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
1 5 10 15

(14) INFORMATION FOR SEQ ID NO: 14:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 471 base pairs
 (B)TYPE: nucleic acid
 (C)STRANDEDNESS: double
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA

(ix)FEATURE:

(A)NAME/KEY: mat peptide

(B)LOCATION: 1..471

(C)IDENTIFICATION METHOD: S

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1				5				10						15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
			20					25						30		
ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
			35					40						45		
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
			50					55						60		
TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
65						70								80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
						85								95		
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
						100								110		
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
														125		
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
														140		
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
145						150								155		

(15) INFORMATION FOR SEQ ID NO: 15:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 10 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1				5				10	

(16) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 471 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: mat peptide
- (B) LOCATION: 1..471
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1			5					10						15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
		20						25					30			
ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
		35					40					45				
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
		50				55					60					
TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
65					70					75				80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
			85						90					95		
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
			100					105					110			
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TCT	GAA	384
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu	
		115					120					125				
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
		130				135					140					
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
145					150					155						

(17) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11464 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii)MOLECULE TYPE: genomic DNA

(vi)ORIGINAL SOURCE:

(A)ORGANISM: human

(G)CELL TYPE: placenta

(ix)FEATURE:

(A)NAME/KEY: 5' UTR

(B)LOCATION: 1..3

(C)IDENTIFICATION METHOD: E

(A)NAME/KEY: leader peptide

(B)LOCATION: 4..82

(C)IDENTIFICATION METHOD: S

(A)NAME/KEY: intron

(B)LOCATION: 83..1453

(C)IDENTIFICATION METHOD: E

(A)NAME/KEY: leader peptide

(B)LOCATION: 1454..1465

(C)IDENTIFICATION METHOD: S

(A)NAME/KEY: intron

(B)LOCATION: 1466..4848

(C)IDENTIFICATION METHOD: E

(A)NAME/KEY: leader peptide

(B)LOCATION: 4849..4865

(C)IDENTIFICATION METHOD: S

(A)NAME/KEY: mat peptide

(B)LOCATION: 4866..4983

(C)IDENTIFICATION METHOD: S

(A)NAME/KEY: intron

(B)LOCATION: 4984..6317

(C)IDENTIFICATION METHOD: E

(A)NAME/KEY: mat peptide

(B)LOCATION: 6318..6451

(C)IDENTIFICATION METHOD: S

(A)NAME/KEY: intron

(B)LOCATION: 6452..11224

(C)IDENTIFICATION METHOD: E

(A)NAME/KEY: mat peptide

(B)LOCATION: 11225..11443

(C)IDENTIFICATION METHOD: S

(A)NAME/KEY: 3' UTR

(B)LOCATION: 11444..11464

(C)IDENTIFICATION METHOD: E

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA	48
Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala	
-35 -30 -25	
ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G GTAAGG CTAATGCCAT	98
Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala	
-20 -15 -10	
AGAACAAATA CCAGGTTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT	158
ATTAAGTGAC TCTTTGTGTC ACCAAATTTTCT ACTGTAATAT TAATGGCTCT TAAAAAATA	218
GTGGACCTCT AGAAATTAAC CACAACATGT CCAAGGTCTC AGCACCTTGT CACACCACGT	278
GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA	338

TCTGTCGCCC AGGCTAGAGT GCAGTGGCAC GATCTCAGCT CACTGCAAGC TCTGCCTCCC 9376
GGGTTCACGC CATTCTCCTG CCTCACCCTC CCAAGCAGCT GGGACTACAG GCGCCTGCCA 9436
CCATGCCCAG CTAATTTTTT GTATTTTTAG TAGAGACGGG GTTTCACCGT GTTAGCCAGG 9496
ATGGTCTCGA TCTCCTGAAC TTGTGATCCG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT 9556
ACAGGCGTGA GCCATCGCAC CCGGCTCAAC TGTAACCTTC TATACTGGTT CATCTTCCCC 9616
TGTAATGTTA CTAGAGCTTT TGAAGTTTTG GCTATGGATT ATTTCTCATT TATACATTAG 9676
ATTTTCAGATT AGTTCCAAAT TGATGCCCCA AGCTTAGGGT CTCTTCCTAA ATTGTATATT 9736
GTAGACAGCT GCAGAAGTGG GTGCCAATAG GGGAAGTAGT TTATACTTTC ATCAACTTAG 9796
GACCCACACT TGTTGATAAA GAACAAAGGT CAAGAGTTAT GACTACTGAT TCCACAACCTG 9856
ATTGAGAAGT TGGAGATAAC CCCGTGACCT CTGCCATCCA GAGTCTTTCA GGCATCTTTG 9916
AAGGATGAAG AAATGCTATT TTAATTTTGG AGGTTTCTCT ATCAGTGCTT AGGATCATGG 9976
GAATCTGTGC TGCCATGAGG CCAAAATTA GTCCAAAACA TCTACTGGTT CCAGGATTAA 10036
CATGGAAGAA CCTTAGGTGG TGCCACATG TTCTGATCCA TCCTGCAAAA TAGACATGCT 10096
GACTAACAG GAAAAGTGCA GGCAGCACTA CCAGTTGGAT AACCTGCAAG ATTATAGTTT 10156
CAAGTAATCT AACCATTTCT CACAAGGCCC TATTCTGTGA CTGAAACATA CAAGAATCTG 10216
CATTTGGCCT TCTAAGGCAG GGCCAGCCA AGGAGACCAT ATTCAGGACA GAAATTCAAG 10276
ACTACTATGG AACTGGAGTG CTTGGCAGGG AAGACAGAGT CAAGGACTGC CAACTGAGCC 10336
AATACAGCAG GCTTACACAG GAACCCAGGG CCTAGCCCTA CAACAATTAT TGGGTCTATT 10396
CACTGTAAGT TTTAATTTCA GGCTCCACTG AAAGAGTAAG CTAAGATTCC TGGCACTTTC 10456
TGTCTCTCTC ACAGTTGGCT CAGAAATGAG AACTGGTCAG GCCAGGCATG GTGGCTTACA 10516
CCTGGAATCC CAGCACTTTG GGAGGCCGAA GTGGGAGGGT CACTTGAGGC CAGGAGTTCA 10576
GGACCAGCTT AGGCAACAAA GTGAGATACC CCCTGACCCC TTCTCTACAA AAATAAATTT 10636
TAAAAATTAG CCAAATGTGG TGGTGATAC TTACAGTCCC AGCTACTCAG GAGGCTGAGG 10696
CAGGGGGATT GCTTGAGCCC AGGAATTCAA GGCTGCAGTG AGCTATGATT TCACCACTGC 10756
ACTTCTGGCT GGGCAACAGA GCGAGACCTT GTCTCAAAGC AAAAAGAAAA AGAACTAGA 10816
ACTAGCCTAA GTTTGTGGGA GGAGGTCATC ATCGTCTTTA GCCGTGAATG GTTATTATAG 10876
AGGACAGAAA TTGACATTAG CCCAAAAAGC TTGTGGTCTT TGCTGGAATC CTAATTATC 10936
TTGAGCAAAT GTGGACACCA CTCAATGGGA GAGGAGAGAA GTAAGCTGTT TGATGTATAG 10996
GGGAAAATA GAGGCCTGGA ACTGAATATG CATCCCATGA CAGGGAGAAT AGGAGATTCTG 11056
GAGTTAAGAA GGAGAGGAGG TCAGTACTGC TGTTTCAGAGA TTTTTTTTAT GTAACCTTTG 11116
AGAAGCAAAA CTACTTTTGT TCTGTTTGGT AATATACTTC AAAACAAACT TCATATATTC 11176
AAATTGTTCA TGTCCTGAAA TAATTAGGTA ATGTTTTTTT CTCTATAG GAA ATG AAT 11233

Glu Met Asn
85

CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC ATC ATA TTC TTT CAG 11281
Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Phe Phe Glu
90 95 100
AGA AGT GTC CCA GGA CAT GAT AAT AAG ATG CAA TTT GAA TCT TCA TCA 11329
Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser
105 110 115
TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG AGA GAC CTT TTT AAA 11377
Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys
120 125 130 135
CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT ATA ATG TTC 11425
Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe
140 145 150
ACT GTT CAA AAC GAA GAC TAGCTATTAA AATTTTCATGC C 11464
Thr Val Gln Asn Glu Asp
155

(18) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 471 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA to mRNA

(vi)ORIGINAL SOURCE:

(A)ORGANISM: mouse

(G)CELL TYPE: liver

(ix)FEATURE:

(A)NAME/KEY: mat peptide

(B)LOCATION: 1..471

(C)IDENTIFICATION METHOD: S

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AAC	TTT	GGC	CGA	CTT	CAC	TGT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn	
1				5					10					15		
GAC	CAA	GTT	CTC	TTC	GTT	GAC	AAA	AGA	CAG	CCT	GTG	TTC	GAG	GAT	ATG	96
Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met	
			20					25					30			
ACT	GAT	ATT	GAT	CAA	AGT	GCC	AGT	GAA	CCC	CAG	ACC	AGA	CTG	ATA	ATA	144
Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile	
		35				40						45				
TAC	ATG	TAC	AAA	GAC	AGT	GAA	GTA	AGA	GGA	CTG	GCT	GTG	ACC	CTC	TCT	192
Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser	
	50					55				60						
GTG	AAG	GAT	AGT	AAA	ATG	TCT	ACC	CTC	TCC	TGT	AAG	AAC	AAG	ATC	ATT	240
Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile	
65				70						75				80		
TCC	TTT	GAG	GAA	ATG	GAT	CCA	CCT	GAA	AAT	ATT	GAT	GAT	ATA	CAA	AGT	288
Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser	
				85					90				95			
GAT	CTC	ATA	TTC	TTT	CAG	AAA	CGT	GTT	CCA	GGA	CAC	AAC	AAG	ATG	GAG	336
Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu	
			100					105					110			
TTT	GAA	TCT	TCA	CTG	TAT	GAA	GGA	CAC	TTT	CTT	GCT	TGC	CAA	AAG	GAA	384
Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Cys	Gln	Lys	Glu	
		115				120						125				
GAT	GAT	GCT	TTC	AAA	CTC	ATT	CTG	AAA	AAA	AAG	GAT	GAA	AAT	GGG	GAT	432
Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp	
	130					135					140					
AAA	TCT	GTA	ATG	TTC	ACT	CTC	ACT	AAC	TTA	CAT	CAA	AGT				471
Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser				
145					150					155						

(19) INFORMATION FOR SEQ ID NO: 19:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 9 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Phe Gly Arg Leu His Cys Thr Thr
1 5

(20) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65 70 75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(21) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60

Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile
65					70					75					80
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
145					150					155					

(22) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
			20					25					30		
Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35				40						45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50				55						60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile
65					70				75					80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
145					150					155					

(23) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
			20					25					30		
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile
65					70					75				80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
145					150					155					

(24) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
			20					25					30		
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ser	Glu	Asn	Lys	Ile
65					70					75				80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
145					150					155					

(25) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
			20					25					30		
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile
65					70					75				80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
145					150					155					

(26) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
			20					25					30		
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile
65					70					75				80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu

WE CLAIM:

1. An osteoclastgenic inhibitory agent, which comprises an interleukin-18 or its functional equivalent.

2. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3 as partial amino acid sequences.

3. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 4 and SEQ ID NO: 5 as partial amino acid sequences.

4. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 6.

5. The inhibitory agent of claim 1, wherein said interleukin-18 is human origin.

6. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 7.

7. The inhibitory agent of claim 1, which is a therapeutic agent for osteoclast-related diseases.

8. The inhibitory agent of claim 1, which contains a protein, buffer, or saccharide as a stabilizer.

9. The inhibitory agent of claim 1, which is in the form of a liquid, paste, or solid.

10. The inhibitory agent of claim 1, which contains 0.000002-100 w/w % of said interleukin-18.

11. A method for treating and/or preventing

osteoclast-related diseases, which comprising administering said inhibitory agent of claim 1 to patients suffering from said diseases at a dose of about 0.5 μ g to 100 mg per shot, 2 to 6 fold a day or 2 to 10 fold a week for one day to one year.

Abstract of the Disclosure

An osteoclastgenic inhibitory agent which comprises an interleukin-18 and/or its functional equivalent. The agent can be arbitrarily used as an ingredient for cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

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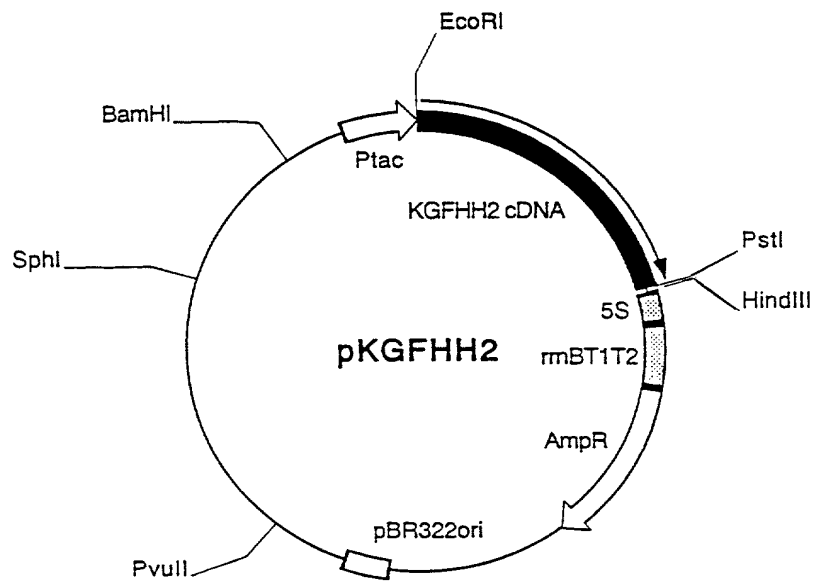


FIG. 1

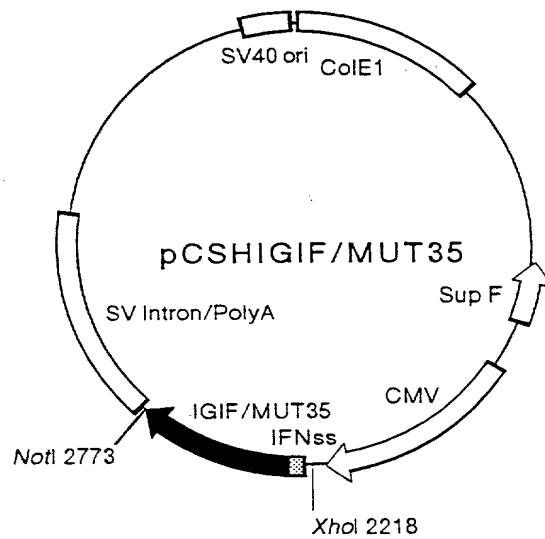


FIG. 2

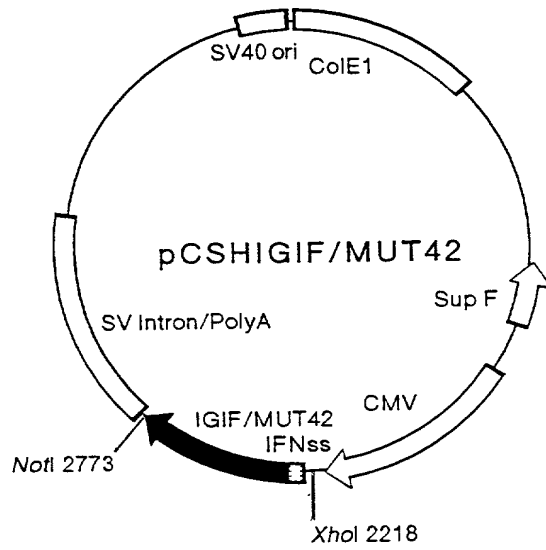


FIG. 3

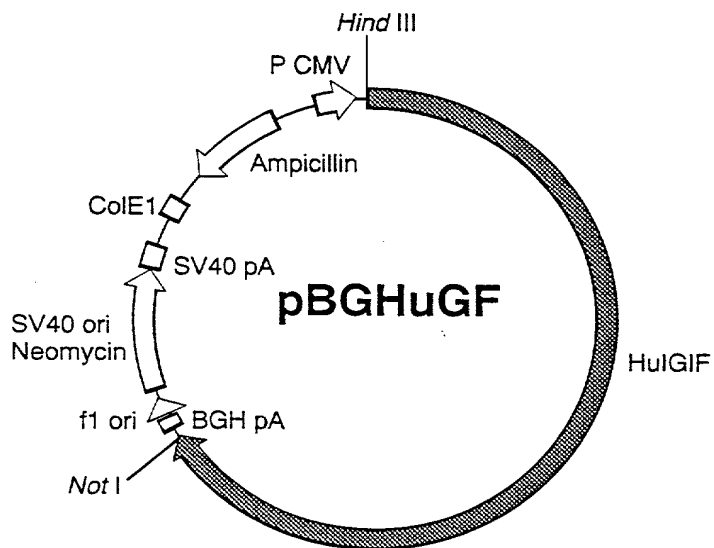


FIG. 4

065220 73999860

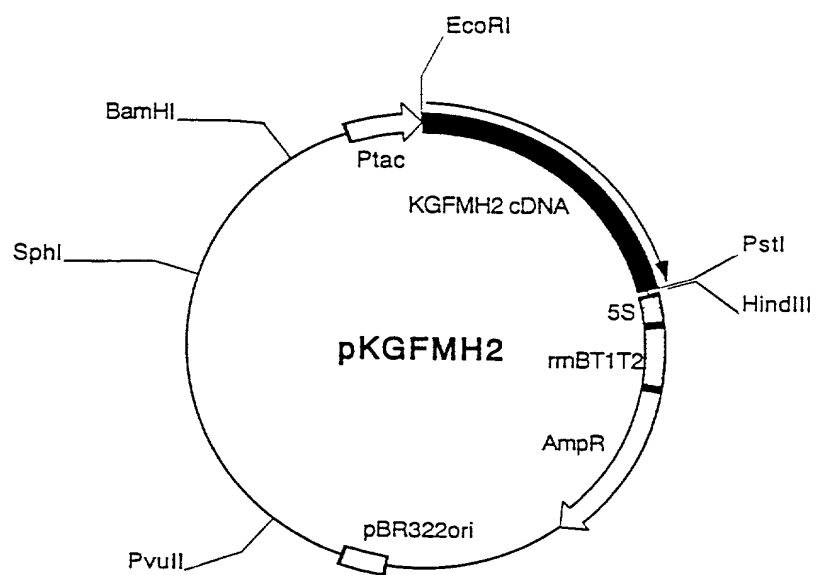


FIG. 5

Page 1 of 2

[x] Original [] Supplemental

Atty. Docket:

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled (insert full title here) **OSTEOCLASTGENIC INHIBITORY AGENT** the specification of which (check one)

[xx] is attached hereto;

[] was filed in the United States under 35 U.S.C. §111 on _____, as USSN _____; or

[] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an intentional (PCT) application, PCT/ _____; filed _____, entry requested on _____; national stage application received USSN _____; §371/§102(e) date _____ (*if known), and was amended on _____ (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

55468/1997	Japan	25th February 1997	[x]	[]
(Number)	(Country)	(Day Month Year Filed)	YES	NO
_____	_____	_____	[]	[]
(Number)	(Country)	(Day Month Year Filed)	YES	NO
_____	_____	_____	[]	[]
(Number)	(Country)	(Day Month Year Filed)	YES	NO

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

_____	_____	_____
(Application Serial NO.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
_____	_____	_____
(Application Serial NO.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SHERIDAN NEIMARK, REG. NO. 20,520 - ROGER L. BROWDY, REG. NO. 25,618 - ANNE M. KORNBAU, REG. NO. 25,884
 NORMAN J. LATKER, REG. NO. 19,963 - IVER P. COOPER, REG. NO. 28,005 - ALLEN C. YUN, REG. NO. 37,971*
 NICK S. BROMER, REG. NO. 33,478 - *Patent Agent

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 (202)628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents named herein to accept and follow instructions from SUMA PATENT OFFICE as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorney or Agent and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents named herein will be so notified by the undersigned.

(S)

Page 2 of 2 **Atty.Docket:**
Title OSTEOCLASTGENIC INHIBITORY AGENT
U.S. Application filed _____, Serial No. _____
PCT Application filed _____, Serial No. _____

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR Matthew Todd GILLMAN		INVENTOR'S SIGNATURE <i>Matthew Gillman</i>	DATE Feb. 15, 1998
RESIDENCE Victoria, Australia		CITIZENSHIP Australian	
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POST OFFICE ADDRESS Department of Medicine, the University of Melbourne and St. Vincent's Institute of Medical Research, 41 Victoria parade, Fitzroy 3065, the Commonwealth of Australia			
FULL NAME OF THIRD JOINT INVENTOR Nobuyuki Udagawa		INVENTOR'S SIGNATURE <i>Nobuyuki Udagawa</i>	DATE Jan. 19, 1998
RESIDENCE Chiba, Japan		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 16-7, 3 chome, Akehara, Kashiwa shi, Chiba, Japan			
FULL NAME OF FOURTH JOINT INVENTOR Masashi Kurimoto		INVENTOR'S SIGNATURE <i>Masashi Kurimoto</i>	DATE Jan. 19, 1998
RESIDENCE Okayama, Japan		CITIZENSHIP Japanese	
POST OFFICE ADDRESS 7-25, 2-chome, Gakunan-cho, Okayama-shi, Okayama, Japan			
FULL NAME OF FIFTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			
FULL NAME OF SIXTH JOINT INVENTOR		INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP	
POST OFFICE ADDRESS			

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.

09030064 022900

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: GILLISPIE, Matthew Todd
HORWOOD, Nicole Joy
UDAGAWA, Nobuyuki
KURIMOTO, Masashi
- (ii) TITLE OF INVENTION: OSTEOCLASTGENIC INHIBITORY AGENT
- (iii) NUMBER OF SEQUENCES: 28
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 - (E) COUNTRY: USA
 - (F) ZIP: 20004
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: Patent In Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 25-FEB-1998
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: JP 55,468/1997
 - (B) FILING DATE: 25-FEB-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: BROWDY, Roger L.
 - (B) REGISTRATION NUMBER: 25,618
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- (ix) TELECOMMUNICATION INFORMATION:
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 - (B) TELEFAX: (202) 737-3528

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asn Asp Gln Val Leu Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Phe Glu Asp Met Thr Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Phe Lys Leu Ile Leu Lys Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Tyr Lys Asp Ser
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Thr Leu Ser Cys
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile

50		55		60
Ser Val Lys Cys Glu	Lys Ile Ser Thr Leu	Ser Cys Glu Asn Lys Ile		
65	70	75	80	
Ile Ser Phe Lys Glu	Met Asn Pro Pro Asp	Asn Ile Lys Asp Thr Lys		
	85	90	95	
Ser Asp Ile Ile Phe	Phe Gln Arg Ser Val	Pro Gly His Asp Asn Lys		
	100	105	110	
Met Gln Phe Glu Ser	Ser Ser Tyr Glu Gly Tyr	Phe Leu Ala Cys Glu		
	115	120	125	
Lys Glu Arg Asp Leu	Phe Lys Ile Leu Lys	Lys Glu Asp Glu Leu		
	130	135	140	
Gly Asp Arg Ser Ile	Met Phe Thr Val Gln	Asn Glu Asp		
145	150	155		

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn	
1 5 10 15	
Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met	
20 25 30	
Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile	
35 40 45	
Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser	
50 55 60	
Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile	
65 70 75 80	
Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser	
85 90 95	
Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu	
100 105 110	
Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu	
115 120 125	
Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp	
130 135 140	
Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser	
145 150 155	

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(G) CELL TYPE: liver

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 1..471

(C) IDENTIFICATION METHOD: E

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT

48

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1				5					10					15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
			20					25					30			
ATG	ACT	GAT	TCT	GAC	TGT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
		35					40					45				
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
		50				55				60						
TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240
Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	
		65			70					75				80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
			85				90						95			
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
			100				105					110				
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
		115					120					125				
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
		130				135				140						
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
145					150					155						

(2) INFORMATION FOR SEQ ID NO: 9:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 11 amino acids
 (B)TYPE: amino acid
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1				5					10	

(2) INFORMATION FOR SEQ ID NO: 10:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 10 amino acids
 (B)TYPE: amino acid
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: C-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp
1				5				10	

(2) INFORMATION FOR SEQ ID NO: 11:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 13 amino acids
 (B)TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 1..471

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1			5					10						15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
		20						25						30		

ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
	35						40					45				
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
	50						55				60					
TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
	65				70					75				80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
			85						90					95		
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
			100					105					110			
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
	115						120					125				
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
	130					135					140					
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
145					150					155						

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1			5					10	

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 471 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: mat peptide
- (B) LOCATION: 1..471
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1			5					10					15			
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
	20					25						30				
ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
	35					40					45					
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192

Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile		
50						55				60							
TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240	
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile		
65					70					75					80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys		
				85					90					95			
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys		
			100					105					110				
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TCT	GAA	384	
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu		
		115					120					125					
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432	
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu		
		130				135					140						
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471	
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp					
145					150					155							

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11464 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (G) CELL TYPE: placenta

(ix) FEATURE:

- (A) NAME/KEY: 5' UTR
- (B) LOCATION: 1..3
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: leader peptide
- (B) LOCATION: 4..82
- (C) IDENTIFICATION METHOD: S
- (A) NAME/KEY: intron
- (B) LOCATION: 83..1453
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: leader peptide
- (B) LOCATION: 1454..1465
- (C) IDENTIFICATION METHOD: S
- (A) NAME/KEY: intron
- (B) LOCATION: 1466..4848
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: leader peptide
- (B) LOCATION: 4849..4865
- (C) IDENTIFICATION METHOD: S
- (A) NAME/KEY: mat peptide
- (B) LOCATION: 4866..4983
- (C) IDENTIFICATION METHOD: S
- (A) NAME/KEY: intron
- (B) LOCATION: 4984..6317
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: mat peptide
- (B) LOCATION: 6318..6451
- (C) IDENTIFICATION METHOD: S
- (A) NAME/KEY: intron
- (B) LOCATION: 6452..11224
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: mat peptide
- (B) LOCATION: 11225..11443

(C) IDENTIFICATION METHOD: S
 (A) NAME/KEY: 3 UTR
 (B) LOCATION: 11444..11464
 (C) IDENTIFICATION METHOD: E

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA	48
Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala	
-35 -30 -25	
ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G GTAAGG CTAATGCCAT	98
Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala	
-20 -15 -10	
AGAACAAATA CCAGGTTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT	158
ATTAAGTGAC TCTTTGTGTC ACCAAATTTT ACTGTAATAT TAATGGCTCT TAAAAAATA	218
GTGGACCTCT AGAAATTAAC CACAACATGT CCAAGGTCTC AGCACCTTGT CACACCACGT	278
GTCTTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA	338
AAATCCCAGT TTTTATGGGA AAATCCCAGT TTTTATGGGA TTTCCATGGG AAAAATCCCA	398
GTACAAAACCT GGGTGCATTG AGGAAATACA ATTTCCCAAA GCAAATGGC AAATTATGTA	458
AGAGATTCTC TAAATTTAGA GTTCCGTGAA TTACACCATT TTATGTAAAT ATGTTTGACA	518
AGTAAAAATT GATTCTTTTT TTTTTTTTCT GTTGCCAGG CTGGAGTGCA GTGGCACAAT	578
CTCTGCTCAC TGCAACCTCC ACCTCCTGGG TTCAAGCAAT TCTCCTGCCT CAGCCTTCTG	638
AGTAGCTGGG ATCAGCGTG CATCCCGCCA TGCTCTGGTA ATTTTTGGGT ATTTTTACTA	698
GAGACAGGGT TTTGGCATGT TGTCCAGGCT GGTCTTGGAC TCCTGATCTC AGATGATCCT	758
CCTGGCTCGG GCTCCCAAAG TGCTGGGATT ACAGGCATGA ACCACCACAC ATGGCCTAAA	818
AATTGATTCT TATGATTAAT CTCCTGTGAA CAATTTGGCT TCATTTGAAA GTTTGCCTTC	878
ATTTGAAACC TTCATTTAAA AGCCTGAGCA ACAAAGTGAG ACCCCATCTC TACAAAAAAC	938
TGCAAAATAT CCTGTGGACA CCTCCTACCT TCTGTGGAGG CTGAAGCAGG AGGATCACTT	998
GAGCCTAGGA ATTTGAGCCT GCAGTGAGCT ATGATCCAC CCCTACACTC CAGCCTGCAT	1058
GACAGTAGAC CCTGACACAC ACACACAAAA AAAAACCTTC ATAAAAAATT ATTAGTTGAC	1118
TTTTCTTAGG TGACTTTCCG TTTAAGCAAT AAATTTAAAA GTAAATCTC TAATTTTAGA	1178
AAATTTATTT TTAGTTACAT ATTGAAATTT TTAACCCCTA GGTTTAAAGT TTATGCTAA	1238
ATTACCTGAG AACACACTAA GTCTGATAAG CTTTATTTTA TGGGCCCTTT GGATGATTAT	1298
ATAATATTCT GATGAAAGCC AAGACAGACC CTTAAACCAT AAAAATAGGA GTTCGAGAAA	1358
GAGGAGTAGC AAAAGTAAAA GCTAGAATGA GATTGAATTC TGAGTCGAAA TACAAAAATT	1418
TACATATTCT GTTTCTCTCT TTTTCCCCCT CTTAG CT GAA GAT GAT G GTAAA	1470
Ala Glu Asp Asp Glu	
-10	
GTAGAAATGA ATTTATTTTT CTTTGCAAAC TAAGTATCTG CTTGAGACAC ATCTATCTCA	1530
CCATTGTCAG CTGAGGAAAA AAAAAAATGG TTCTCATGCT ACCAATCTGC CTTCAAAGAA	1590
ATGTGGACTC AGTAGCACAG CTTTGGGAATG AAGATGATCA TAAGAGATAC AAAGAAGAAC	1650
CTCTAGCAAA AGATGCTTCT CTATGCCTTA AAAAATCTC CAGCTCTTAG AATCTACAAA	1710
ATAGACTTTG CCTGTTTCAT TGGTCTTAAG ATTAGCATGA AGCCATGGAT TCTGTTGTAG	1770
GGGGAGCGTT GCATAGGAAA AAGGGATTGA AGCATTAGAA TTGTCCAAAA TCAGTAACAC	1830
CTCCTCTCAG AAATGCTTTG GGAAGAAGCC TGGAAAGTTT CGGGTTGGTG GTGGGTGGG	1890
GCAGAAAATT CTGGAAGTAG AGGAGATAGG AATGGGTGGG GCAAGAAGAC CACATTGAGA	1950
GGCCAAAAGC TGAAAGAAAC CATGGCATT ATGATGAATT CAGGGTAATT CAGAATGGAA	2010
GTAGAGTAGG AGTAGGAGC TGGTGAGAGG AGCTAGATG ATAAACAGGG TGTAAGACAA	2070
GACGTTCTCT CACCCCAAGA TGTGAAATTT GGTGATTTATC TTGGAGATAA TAGGGTTAAT	2130
TAAGCACAAT ATGTATTAGC TAGGGTAAAG ATTAGTTTGT TGTAACAAAG ACATCCAAAG	2190
ATACAGTAGC TGAATAAGAT AGAGAATTTT TCTCTCAAAG AAAGTCTAAG TAGGCAGCTC	2250
AGAAGTAGTA TGGCTGGAAG CAACCTGATG ATATTGGGAC CCCCACCTT CTTCACTCTT	2310
GTACCCATCA TCCCCTAGTT GTTGATCTCA CTCACATAGT TGAAAATCAT CATACTTCTT	2370
GGGTTTCATAT CCCAGTTATC AAGAAAGGGT CAAGAGAAGT CAGGCTCATT CTTTCAAAG	2430
ACTCTAATTG GAAGTTAAAC ACATCAATCC CCCTCATATT CCATTGACTA GAATTTAATC	2490
ACATGGCCAC ACCAAGTGCA AGGAAATCTG GAAAATATAA TCTTTATTCC AGGTAGCCAT	2550
ATGACTCTTT AAAATTCAGA AATAATATAT TTTTAAATA TCATTCTGGC TTTGGTATAA	2610
AGAATTGATG GTGTGGGGTG AGGAGGCCAA AATTAAGGGT TGAGAGCCTA TTATTTTAGT	2670
TATTACAAGA AATGATGGTG TCATGAATTA AGGTAGACAT AGGGGAGTGC TGATGAGGAG	2730
CTGTGAATGG ATTTTAGAAA CACTTGAGAG AATCAATAGG ACATGATTTA GGGTTGGATT	2790
TGGAAAGGAG AAGAAAGTAG AAAAGATGAT GCCTACATTT TTCATTAGG CAATTTGTAC	2850
CATTCAAGTA AAGAGGAAAC ACAGGAGGAA GAGCAGTTT TGGTGTATAC AAAGAGGAGG	2910
ATGGATGACG CATTTTCGTTT TGGATCTGAG ATGCTCTGGG AACGTCCTAG TGGAGATGTC	2970
CACAACTCT TCTACATGTG GTTCTGAGTT CAGGACACAG ATTTGGGCTG GAGATAGAGA	3030
TATTGTAGGC TTATACATAG AAATGGCATT TGAATCTATA GAGATAAAAA GACACATCAG	3090
AGGAAATGTG TAAAGTGAGA GAGGAAAAGC CAAGTACTGT GCTGGGGGGA ATACCTACAT	3150
TTAAAGGATG CAGTAGAAAG AAGCTAATAA ACAACAGAGA GCAGACTAAC CAAAAGGGGA	3210

GAAGAAAAAC	CAAGAGAATT	CCACCGACTC	CCAGGAGAGC	ATTTCAAGAT	TGAGGGGATA	3270
GGTGTGTGT	TGAATTTTGC	AGCCTTGAGA	ATCAAGGGCC	AGAACACAGC	TTTTAGATTT	3330
AGCAACAAGG	AGTTTGGTGA	TCTCAGTGAA	AGCAGCTTGA	TGGTGAAATG	GAGGCAGAGG	3390
CAGATTGCAA	TGAGTGAAAC	AGTGAATGGG	AAGTGAAGAA	ATGATACAGA	TAATTCCTTG	3450
TAAAAGCTTG	GCTGTAAAA	GGAGGAGAGA	AACAAGACTA	GCTGCAAAGT	GAGATTGGGT	3510
TGATGGAGCA	GTTTTAAATC	TCAAAATAAA	GAGCTTTGTG	CTTTTTTGAT	TATGAAAATA	3570
ATGTGTAAAT	TGTAACATA	TGAGGCAATG	AAAAAAGATA	ATAATATGAA	AGATAAAAAAT	3630
ATAAAAAACCA	CCCAGAAATA	ATGATAGCTA	CCATTTTGAT	ACAATATTTT	TACACTCCTT	3690
TCTATGTATA	TATACAGACA	CAGAAATGCT	TATATTTTTA	TTAAAAGGGA	TTGTACTATA	3750
CCTAAGCTGC	TTTTTCTAGT	TAGTGATATA	TATGGACATC	TCTCCATGGC	AACGAGTAAT	3810
TGCAGTTATA	TTAAGTTCAT	GATATTTTAC	AATAAGGGCA	TATCTTTGCC	CTTTTTATTT	3870
AATCAATTCT	TAATTGGTGA	ATGTTTGTG	CCAGTTTGTG	GTTGTTATTA	ACAATGTTCC	3930
CATAAGCATT	CCTGTACACC	AATGTTTACA	CATTTGTCTG	ATTTTTTCTT	CAGGATAAAA	3990
CCCAGGAGGT	AGAATTGCTG	GGTTGATAGA	AGAGAAAGGA	TGATTGCCAA	ATTAAAGCTT	4050
CAGTAGAGGG	TACATGCCGA	GCACAAATGG	GATCAGCCCT	AGATACCAGA	AATGGCATT	4110
TCTCATTTCC	CCTTGGGACA	AAAGGGAGAG	AGGCAATAAC	TGTGCTGCCA	GAGTTAAATT	4170
TGTACGTGGA	GTAGCAGGAA	ATCATTGTCT	GAAAATGAAA	ACAGAGATGA	TGTTGTAGAG	4230
GTCTGAAGA	GAGCAAAGAA	AATTTGAAAT	TGCGGCTATC	AGCTATGGAA	GAGAGTGCTG	4290
AACTGGAAAA	CAAAAGAAGT	ATTGACAATT	GGTATGCTTG	TAATGGCACC	GATTTGAACG	4350
CTTGTGCCAT	TGTTACCAG	CAGCACTCAG	CAGCCAAGTT	TGGAGTTTTG	TAGCAGAAAG	4410
ACAAATAAGT	TAGGGATTTA	ATATCCTGGC	CAAATGGTAG	ACAAAATGAA	CTCTGAGATC	4470
CAGCTGCACA	GGGAAGGAAG	GGGAAGCGGG	AAGAGGTTAG	ATAGGAAATA	CAAGAGTCAG	4530
GAGACTGGAA	GATGTTGTGA	TATTTAAGAA	CACATAGAGT	TGGAGTAAAA	GTGTAAGAAA	4590
ACTAGAAGGG	TAAGAGACCG	GTCAGAAAGT	AGGCTATTTG	AAGTTAACAC	TTCAGAGGCA	4650
GAGTAGTTCT	GAATGGTAAC	AAGAAATTGA	GTGTGCCTTT	GAGAGTAGGT	TAAAAACAA	4710
TAGGCAACTT	TATTGTAGCT	ACTTCTGGAA	CAGAAGATTG	TCATTAAATG	TTTTAGAAAA	4770
CTAAAATATA	TAGCATACTT	ATTTGTCAAT	TAACAAAGAA	ACTATGTATT	TTTAAATGAG	4830
ATTTAATGTT	TATTGTAG	AA AAC CTG	GAA TCA GAT	TAC TTT GGC	AAG CTT	4880
		Glu Asn Leu Glu Ser Asp	Tyr Phe Gly Lys Leu			
		-5	1	5		
GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT	GAC CAA GTT CTC TTC					4928
Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn	Asp Gln Val Leu Phe					
		10	15	20		
ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT	ATG ACT GAT TCT GAC					4976
Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp	Met Thr Asp Ser Asp					
		25	30	35		
TGT AGA G GTATTTTTT TTAATTCGCA AACATAGAAA	TGACTAGCTA CTTCTTCCCA					5032
Cys Arg Asp						
		40				
TTCTGTTTTA CTGCTTACAT TGTTCCGTGC TAGTCCCAAT	CCTCAGATGA AAAGTCACAG					5092
GAGTGACAAT AATTTTCACTT ACAGGAAACT TTATAAGGCA	TCCACGTTTT TTAGTTGGGG					5152
TAAAAAATTG GATACAATAA GACATTGCTA GGGGTCATGC	CTCTCTGAGC CTGCCTTTGA					5212
ATCACCAATC CCTTTATTGT GATTGCATTA ACTGTTTAAA	ACCTCTATAG TTGGATGCTT					5272
AATCCCTGCT TGTTACAGCT GAAAATGCTG ATAGTTTACC	AGGTGTGGTG GCATCTATCT					5332
GTAATCCTAG CTACTTGGGA GGCTCAAGCA GGAGGATTGC	TTGAGGCCAG GACTTTGAGG					5392
CTGTAGTACA CTGTGATCGT ACCTGTGAAT AGCCACTGCA	CTCCAGCCTG GGTGATATAC					5452
AGACCTTGTC TCTAAAATTA AAAAAAAAAA AAAAAAAAAAC	CTTAGGAAAG GAAATGATC					5512
AAGTCTACTG TGCCTTCCAA AACATGAATT CCAAAATATCA	AAGTTAGGCT GAGTTGAAGC					5572
AGTGAATGTG CATTCTTTAA AAATACTGAA TACTTACCTT	AACATATATT TTAAATATTT					5632
TATTTAGCAT TTAAGAGTTA AAAACAATCT TTTAGAATTC	ATATCTTTAA AATACTCAAA					5692
AAAGTTGCAG CGTGTGTGTT GTAATACACA TTAAACTGTG	GGGTTGTTTG TTTGTTTGAG					5752
ATGCAGTTTC ACTCTGTCAC CCAGGCTGAA GTGCAGTGCA	GTGCAGTGGT GTGATCTCGG					5812
CTCACTACAA CCTCCACCTC CCACGTTCAA GCGATTCTCA	TGCCCTCAGT TCCCGAGTAG					5872
GTGGGATTAC AGGCATGCAC CACTTACACC CGGCTAATTT	TTGTATTTTT AGTAGAGCTG					5932
GGGTTTCACC ATGTTGGCCA GGCTGGTCTC AAACCCCTAA	CCTCAAGTGA TCTGCCTGCC					5992
TCAGCCTCCC AAACAAACAA ACAACCCAC AGTTTAATAT	GTGTTACAAC ACACATGCTG					6052
CAACTTTTAT GAGTATTTTA ATGATATAGA TTATAAAGG	TTGTTTTTAA CTTTTAAATG					6112
CTGGGATTAC AGGCATGAGC CACTGTGCCA GGCTGAACT	GTGTTTTTAA AAATGTCTGA					6172
CCAGCTGTAC ATAGTCTCCT GCAGACTGGC CAAGTCTCAA	AGTGGGAACA GGTGTATTAA					6232
GGACTATCCT TTGGTTAAAT TTCCGCAAAT GTTCCTGTGC	AAGAATCTT CTAAGTAGAG					6292
TTCTCATTTA TTATATTTAT TTCAG AT AAT GCA CCC	CGG ACC ATA TTT ATT					6343
	Arg Asn Ala Pro					
	40					
ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT	ATG GCT GTA ACT ATC					6391
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly	Met Ala Val Thr Ile					
		50	55	60		
TCT GTG AAG TGT GAG AAA ATT TCA ACT CTC TCC	TGT GAG AAC AAA ATT					6439

Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	
65					70					75				80		
ATT	TCC	TTT	AAG	GTAAG	ACTGAGCCTT	ACTTTGT	TTTT	CAATCATGTT	AATATAATCA	6496						
Ile	Ser	Phe	Lys													
ATATAATTAG	AAATAAACA	TTATTTCTAA	TGTTAATATA	AGTAATGTAA	TTAGAAAAC	6556										
CAAAATATCCT	CAGACCAACC	TTTTGTCTAG	AACAGAAATA	ACAAGAAGCA	GAGAACCATT	6616										
AAAGTGAATA	CTTACTAAAA	ATTATCAAAC	TCTTTACCTA	TTGTGATAAT	GATGGTTTTT	6676										
CTGAGCCTGT	CACAGGGGAA	GAGGAGATAC	AACACTTGTT	TTATGACCTG	CATCTCCTGA	6736										
ACAATCAGTC	TTTATACAAA	TAATAATGTA	GAATACATAT	GTGAGTTATA	CATTTAAGAA	6796										
TAACATGTGA	CTTTCCAGAA	TGAGTTCTGC	TATGAAGAAT	GAAGCTAATT	ATCCTTCTAT	6856										
ATTTCTACAC	CTTTGTAAAT	TATGATAATA	TTTTAATCCC	TAGTTGTTTT	GTTGCTGATC	6916										
CTTAGCCTAA	GTCTTAGACA	CAAGCTTCAG	CTTCCAGTTG	ATGTATGTTA	TTTTAATGT	6976										
TAATCTAATT	GAATAAAAGT	TATGAGATCA	GCTGTAAAAG	TAATGCTATA	ATTATCTTCA	7036										
AGCCAGGTAT	AAAGTATTTT	TGGCCTCTAC	TTTTTCTCTA	TTATTCTCCA	TTATTATTCT	7096										
CTATTATTTT	TCTCTATTTT	CTCCATTATT	GTTAGATAAA	CCACAATTAA	CTATAGCTAC	7156										
AGACTGAGCC	AGTAAGAGTA	GCCAGGGATG	CTTACAAATT	GGCAATGCTT	CAGAGGAGAA	7216										
TTCCATGTCA	TGAAGACTCT	TTTTGAGTGG	AGATTTGCCA	ATAAATATCC	GCTTTCATGC	7276										
CCACCCAGTC	CCCAGTAAA	GACAGTTAGG	ATATGACCTT	AGTGAAGGTA	CCAAGGGGCA	7336										
ACTTGGTAGG	GAGAAAAAAG	CCACTCTAAA	ATATAATCCA	AGTAAGAACA	GTGCATATGC	7396										
AACAGATACA	GCCCCCAGAC	AAATCCCTCA	GCTATCTCCC	TCCAACCAGA	GTGCCACCCC	7456										
TTCAGGTGAC	AATTTGGAGT	CCCCATTCTA	GACCTGACAG	GCAGCTTAGT	TATCAAAATA	7516										
GCATAAGAGG	CCTGGGATGG	AAGGGTAGGG	TGGAAAGGGT	TAAGCATGCT	GTTACTGAAC	7576										
AACATAATTA	GAAGGGAAGG	AGATGGCCAA	GCTCAAGCTA	TGTGGGATAG	AGGAAAACTC	7636										
AGCTGCAGAG	GCAGATTTCAG	AAACTGGGAT	AAGTCCGAAC	CTACAGGTGG	ATTCTTGTGG	7696										
AGGGAGACTG	GTGAAAATGT	TAAGAAGATG	GAAATAATGC	TTGGCACTTA	GTAGGAACTG	7756										
GGCAAATCCA	TATTTGGGGG	AGCCTGAAGT	TTATTCAATT	TTGATGGCCC	TTTTAAATAA	7816										
AAAGAATGTG	GCTGGGCGTG	GTGGCTCACA	CCTGTAATCC	CAGCACTTTG	GGAGGCCGAG	7876										
GGGGGCGGAT	CACCTGAAGT	CAGGAGTTCA	AGACCAGCCT	GACCAACATG	GAGAAACCCC	7936										
ATCTCTACTA	AAAATACAAA	ATTAGCTGGG	CGTGGTGGCA	TATGCCCTGTA	ATCCCAGCTA	7996										
CTCGGGAGGC	TGAGGCAGGA	GAATCTTTTT	AACCCGGGAG	GCAGAGGTTG	CGATGAGCCT	8056										
AGATCGTGCC	ATTGCACTCC	AGCCTGGGCA	ACAAGAGCAA	AACTCGGTCT	CAAAAAAAAA	8116										
AAAAAAGG	TGAAATTAAC	CAAAGGCATT	AGCTTAATAA	TTTAATACTG	TTTTTAAGTA	8176										
GGGCGGGGGG	TGGCTGGAAG	AGATCTGTGT	AAATGAGGGA	ATCTGACATT	TAAGCTTCAT	8236										
CAGCATCATA	GCAAATCTGC	TTCTGGAAGG	AACTCAATAA	ATATTAGTTG	GAGGGGGGGA	8296										
GAGAGTGAGG	GGTGGACTAG	GACCAGTTTT	AGCCCTTGTC	TTTAATCCCT	TTTCTTGCCA	8356										
CTAATAAGGA	GTTTAGCAGT	GGTTATAAAA	GTGGCTTAGG	TTCTAGATAA	TAAGATACAA	8416										
CAGGCCAGGC	ACAGTGGCTC	ATGCCATATA	TCCCAGCACT	TTGGGAGGGC	AAGGCGAGTG	8476										
TCTCACTTGA	GATCAGGAGT	TCAAGACCAG	CCTGGCCAGC	ATGGCGATAC	TCTGTCTCTA	8536										
CTAAAAAATA	TACAAAAATT	AGCCAGGCAT	GGTGGCATGC	ACCTGTAATC	CCAGCTACTC	8596										
GTGAGCCTGA	GGCAGAAGAA	TCGCTTGAA	CAGGAGGTG	TAGGCTGCAG	TGAGCTGAGA	8656										
TCGCACCACT	GCACTCCAGC	CTGGGCGACA	GAATGAGACT	TTGTCTCAAA	AAAAAGAAAA	8716										
GATACAACAG	GCTACCCTTA	TGTGCTCACC	TTTCACTGTT	GATTACTAGC	TATAAAGTCC	8776										
TATAAAGTTC	TTTGGTCAAG	AACCTTGACA	ACACTAAGAG	GGATTGCTT	TGAGAGGTTA	8836										
CTGTGAGAGT	CTGTTTTCATA	TATATACATA	TACATGTATA	TATGTATCTA	TATCCAGGCT	8896										
TGGCCAGGGT	TCCCTCAGAC	TTTCCAGTGC	ACTTGGGAGA	TGTTAGGTCA	ATATCAAGTT	8956										
TCCCTGGATT	CAGATTCAAC	CCCTTCTGAT	GTAAAAAATA	AAAAAATAAT	GAAAGAAATC	9016										
CCTTTCCCTT	TGGAGCACTC	AAGTTTCACC	AGGTGGGGCT	TTCCAAGTTG	GGGGTTCTCC	9076										
AAGGTCATTG	GGATTGCTTT	CACATCCATT	TGCTATGTAC	CTTCCCTATG	ATGGCTGGGA	9136										
GTGGTCAACA	TCAAAACTAG	GAAAGCTACT	GCCCAAGGAT	GTCTTACCT	CTATTCTGAA	9196										
ATGTGCAATA	AGTGTGATTA	AAGAGATTGC	CTGTTCTACC	TATCCCACT	CTCGCTTTCA	9256										
ACTGTAACCT	TCTTTTTTTC	TTTTTTTCTT	TTTTTCTTTT	TTTTTGAAAC	GGAGTCTCGC	9316										
TCTGTGCGCC	AGGCTAGAGT	GCAGTGGCAC	GATCTCAGCT	CACTGCAAGC	TCTGCCTCCC	9376										
GGGTTACAGC	CATTCTCCTG	CCTCACCTC	CCAAGCAGCT	GGGACTACAG	GCGCCTGCCA	9436										
CCATGCCCAG	CTAATTTTTT	GTATTTTTAG	TAGAGACGGG	GTTTCACCGT	GTTAGCCAGG	9496										
ATGGTCTCGA	TCTCCTGAAC	TTGTGATCCG	CCGCGCTCAG	CCTCCCAAAG	TGCTGGGATT	9556										
ACAGGCGTGA	GCCATCGCAC	CCGGCTCAAC	TGTAACTTTC	TATACTGGTT	CATCTTCCCC	9616										
TGTAATGTTA	CTAGAGCTTT	TGAAGTTTTG	GCTATGGATT	ATTTCTCATT	TATACATTAG	9676										
ATTTGAGATT	AGTTCCAAAT	TGATGCCAC	AGCTTAGGGT	CTCTTCCTAA	ATTGTATATT	9736										
GTAGACAGCT	GCAGAAGTGG	GTGCCAATAG	GGGAAC TAGT	TTATACTTTC	ATCAACTTAG	9796										
GACCCACACT	TGTTGATAAA	GAACAAAGGT	CAAGAGTTAT	GACTACTGAT	TCCACAACCTG	9856										
AATTGAGAAGT	TGGAGATAAC	CCCGTGACCT	CTGCCATCCA	GAGTCTTTCA	GGCATCTTTG	9916										
AAGGATGAAG	AAATGCTATT	TTAATTTTGG	AGGTTTCTCT	ATCAGTGCTT	AGGATCATGG	9976										
GAATCTGTGC	TGCCATGAGG	CCAAAATTAA	GTCCAAAACA	TCTACTGGTT	CCAGGATTAA	10036										
CATGGAAGAA	CCTTAGGTGG	TGCCACATG	TTCTGATCCA	TCCTGCAAAA	TAGACATGCT	10096										
GCACTAACAG	GAAAAGTGCA	GGCAGCACTA	CCAGTTGGAT	AACCTGCAAG	ATTATAGTTT	10156										
CAAGTAATCT	AACCATTCT	CACAAGGCC	TATTCGTGA	CTGAAACATA	CAAGAATCTG	10216										
CATTTGGCCT	TCTAAGGCAG	GGCCAGCCA	AGGAGACCAT	ATTGAGGACA	GAAATTCAAG	10276										

ACTACTATGG	AACTGGAGTG	CTTGGCAGGG	AAGACAGAGT	CAAGGACTGC	CAACTGAGCC	10336
AATACAGCAG	GCTTACACAG	GAACCCAGGG	CCTAGCCCTA	CAACAATTAT	TGGGTCTATT	10396
CACTGTAAAGT	TTTAATTTCA	GGCTCCACTG	AAAGAGTAAG	CTAAGATTCC	TGGCACTTTC	10456
TGTCCTCTCTC	ACAGTTGGCT	CAGAAATGAG	AACTGGTCAG	GCCAGGCATG	GTGGCTTACA	10516
CCTGGAATCC	CAGCACTTTG	GGAGGCCGAA	GTGGGAGGGT	CACCTTGAGGC	CAGGAGTTCA	10576
GGACCAGCTT	AGGCAACAAA	GTGAGATACC	CCCTGACCCC	TTCTCTACAA	AAATAAATTT	10636
TAAAAATTAG	CCAAATGTGG	TGGTGTATAC	TTACAGTCCC	AGCTACTCAG	GAGGCTGAGG	10696
CAGGGGGATT	GCTTGAGCCC	AGGAATTCAA	GGCTGCAGTG	AGCTATGATT	TCACCACTGC	10756
ACTTCTGGCT	GGGCAACAGA	GCGAGACCCT	GTCTCAAAGC	AAAAAGAAAA	AGAAACTAGA	10816
ACTAGCCTAA	GTTTGTGGGA	GGAGGTCATC	ATCGTCTTTA	GCCGTGAATG	GTTATTATAG	10876
AGGACAGAAA	TTGACATTAG	CCCAAAAAGC	TTGTGGTCTT	TGCTGGAAGT	CTACTTAATC	10936
TTGAGCAAAT	GTGGACACCA	CTCAATGGGA	GAGGAGAGAA	GTAAGCTGTT	TGATGTATAG	10996
GGGAAAACTA	GAGGCCTGGA	ACTGAATATG	CATCCCATGA	CAGGGAGAAT	AGGAGATTCC	11056
GAGTTAAGAA	GGAGAGGAGG	TCAGTACTGC	TGTTTCAGAGA	TTTTTTTTTAT	GTAAGTCTTG	11116
AGAAGCAAAA	CTACTTTTGT	TCTGTTTGGT	AATATAC TTC	AAAACAAACT	TCATATATTC	11176
AAATTGTTCA	TGTCCTGAAA	TAATTAGGTA	ATGTTTTTTTT	CTCTATAG	GAA ATG AAT	11233

Glu Met Asn
85

CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	AGT	GAC	ATC	ATA	TTC	TTT	CAG	11281
Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	Ser	Asp	Ile	Ile	Phe	Phe	Glu	
		90					95					100				
AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	ATG	CAA	TTT	GAA	TCT	TCA	TCA	11329
Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	Met	Gln	Phe	Glu	Ser	Ser	Ser	
		105					110				115					
TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	AAA	GAG	AGA	GAC	CTT	TTT	AAA	11377
Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	Lys	Glu	Arg	Asp	Leu	Phe	Lys	
		120				125				130					135	
CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	GGG	GAT	AGA	TCT	ATA	ATG	TTC	11425
Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	Gly	Asp	Arg	Ser	Ile	Met	Phe	
			140						145					150		
ACT	GTT	CAA	AAC	GAA	GAC	TAGCTATTAA	AATTTTCATGC	C								11464
Thr	Val	Gln	Asn	Glu	Asp											
			155													

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 471 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (G) CELL TYPE: liver

(ix) FEATURE:

- (A) NAME/KEY: mat peptide
- (B) LOCATION: 1..471
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AAC	TTT	GGC	CGA	CTT	CAC	TGT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn	
1				5					10					15		
GAC	CAA	GTT	CTC	TTC	GTT	GAC	AAA	AGA	CAG	CCT	GTG	TTC	GAG	GAT	ATG	96
Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met	
			20					25					30			
ACT	GAT	ATT	GAT	CAA	AGT	GCC	AGT	GAA	CCC	CAG	ACC	AGA	CTG	ATA	ATA	144
Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile	
		35					40					45				
TAC	ATG	TAC	AAA	GAC	AGT	GAA	GTA	AGA	GGA	CTG	GCT	GTG	ACC	CTC	TCT	192
Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser	

50	55	60	
GTG AAG GAT AGT AAA ATG TCT ACC CTC TCC TGT AAG AAC AAG ATC ATT			240
Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile			
65	70	75	80
TCC TTT GAG GAA ATG GAT CCA CCT GAA AAT ATT GAT GAT ATA CAA AGT			288
Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser			
85	90	95	
GAT CTC ATA TTC TTT CAG AAA CGT GTT CCA GGA CAC AAC AAG ATG GAG			336
Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu			
100	105	110	
TTT GAA TCT TCA CTG TAT GAA GGA CAC TTT CTT GCT TGC CAA AAG GAA			384
Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu			
115	120	125	
GAT GAT GCT TTC AAA CTC ATT CTG AAA AAA AAG GAT GAA AAT GGG GAT			432
Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp			
130	135	140	
AAA TCT GTA ATG TTC ACT CTC ACT AAC TTA CAT CAA AGT			471
Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser			
145	150	155	

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Phe Gly Arg Leu His Cys Thr Thr
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 157 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn	
1 5 10 15	
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp	
20 25 30	
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile	
35 40 45	
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile	
50 55 60	
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile	
65 70 75 80	
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys	
85 90 95	
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys	
100 105 110	
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu	
115 120 125	
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu	
130 135 140	
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp	
145 150 155	

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65 70 75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65 70 75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
115 120 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 157 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1      5      10
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
      20      25      30
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
      35      40      45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
      50      55      60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
      65      70      75      80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
      85      90      95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
      100      105      110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
      115      120      125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
      130      135      140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145      150      155

```

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1      5      10
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
      20      25      30
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
      35      40      45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
      50      55      60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile
      65      70      75      80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
      85      90      95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
      100      105      110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
      115      120      125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
      130      135      140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145      150      155

```

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
65 70 75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
65 70 75 80
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
85 90 95
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
100 105 110
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
115 120 125
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn

1				5				10					15				
Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met		
			20					25					30				
Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile		
		35					40					45					
Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser		
	50					55					60						
Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile		
	65				70					75				80			
Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser		
				85					90					95			
Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu		
			100					105					110				
Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Cys	Gln	Lys	Glu		
	115					120						125					
Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp		
	130					135					140						
Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser					
	145				150					155							

(2) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 157 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn		
1				5					10					15			
Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met		
			20					25					30				
Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile		
		35					40					45					
Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser		
	50					55					60						
Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile		
	65				70					75				80			
Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser		
				85					90					95			
Asp	Leu	Ile	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu			
			100				105					110					
Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Ser	Gln	Lys	Glu		
	115					120						125					
Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp		
	130					135					140						
Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser					
	145				150					155							